EVALUATION AND CHARACTERISATION OF BIOADHESIVE SUPPOSITORIES FORMULATED USING COMMERCIAL HYDROGENATED PALM KERNEL STEARIN

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ABSTRACT

Rectal route of drug administration is particularly useful when patients cannot tolerate orally yet are unable to receive parenteral injections. Furthermore, studies have shown that it may be possible to circumvent first pass metabolism if absorption was localised in lower rectum. This is theoretically achievable if suppositories were bioadhesive.

The aim of this thesis was to evaluate two types of commercial hydrogenated palm kernel stearin (HPKS) namely ChocExa (CE) and Supersocolate Special™ (SS) as base candidates for bioadhesive suppositories in comparison to cocoa butter (CB). Robustness of these bases during suppository manufacturing was compared using both DSC-simulated and extemporaneous methods. Diclofenac sodium (DcNa) which undergoes extensive first pass metabolism was selected as model drug. Suppositories containing 50 mg DcNa and 1-5 %w/w bioadhesive polymers manufactured using CB, CE and SS as base were evaluated in terms of physical properties, drug release, bioadhesive properties as well as stability under different storage conditions. The bioadhesive polymers used were Carbopol® 974P NF (CBP), hydroxypropyl methylcellulose 2910 (HPMC), poly(vinylpyrrolidone) K30 (PVP) and carboxymethyl chitosan (CMCTS). Two self-fabricated methods using the texture analyser (tensile and shear stress) were developed to study bioadhesion of suppositories against porcine colon mucosa under simulated rectal conditions.

Physical characterisation found that CE and SS were comparable to CB in terms of thermal profile, solid fat content (SFC), pH, viscosity and displacement values (DV) but with added advantages of reduced polymorphism and less stringent manufacturing parameters. Solidification of CB melt into suppositories was highly dependent on the

maximum heating temperature (T_{max}) and cooling rate (C_{rate}). HPKS on the other hand were more robust, as long as it is completely molten HPKS would solidify into stable β' polymorph; while cooling rates did not affect crystallisation. All the bioadhesive suppositories melt between 32.5-35.5 °C. Addition of CBP decreased rate and extent of DcNa release in a concentration dependent manner, resulting in bi-exponential first-order kinetics release pattern. The other bioadhesive polymers had minimal impact on DcNa release. The tensile method to study bioadhesion found that bioadhesive properties decreased in the order of PVP > CBP > CMCTS > HPMC while the shear method PVP > CMCTS > CBP = HPMC. In both instances, HPMC showed poor bioadhesion with limited benefit in development of bioadhesive suppositories. Formulations containing 5 %w/w PVP and CMCTS were selected for subsequent stability assessment based on considerations of complete DcNa release and good bioadhesive properties. These suppositories required refrigeration as suppositories stored for 200 days at room temperature (24.5 ± 2.5 °C; RH 58 ± 5 %) showed graininess and loss of surface glossiness, increased melting point, possible triacylglycerol (TAG) separation, higher SFC at 37 °C, prolonged softening times and decreased amount of DcNa release. These changes were unfavourable for suppositories and may lead to ineffective treatment. Generally, SS suppositories subjected to accelerated ageing released DcNa more efficiently than CE.

Although both HPKS were suitable suppository base substitutes of CB, SS provided superior stability in terms of resistance to depression of DcNa release. PVP on the other hand conferred the best bioadhesive properties among all polymers evaluated. Thus, SS suppositories incorporated with 50 mg DNa and 5 %w/w PVP may be a potential candidate for further development.

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ANOVA	Analysis of variance
AIC	Akaike information criterion
CB	Cocoa butter
CBP	Carbopol 974P NF
CBS	Cocoa butter substitute
CE	Chocexa
CMCTS	Carboxymethyl chitosan
DcNa	Diclofenac sodium
DE	Dissolution efficiency
DV	Displacement value
DSC	Differential scanning calorimetry
f_1	Difference factor
f_2	Similarity factor
F_{max}	Peak force of detachment
FTIR	Fourier transform infrared
GIT	Gastrointestinal tract
HPKS	Hydrogenated palm kernel stearin
HPMC	Hydroxypropyl methylcellulose
HSD	Honestly significant test
IM	Intramuscular
IV	Intravenous
KBr	Potassium bromide
MDT	Mean dissolution time

MPOB	Malaysian Palm Oil Board
MSE	Mean square error
MW	Molecular weight
NSAID	Nonsteroidal anti-inflammatory drug
PEG	Polyethylene glycol
PKO	Palm kernel oil
PKS	Palm kernel stearin
p-NMR	Pulsed nuclear magnetic resonance
PVP	Poly(vinylpyrrolidone)
r^2	Coefficient of determination
RH	Relative humidity
R_t	Reference formulation
SCF	Simulated colonic fluid
SD	Standard deviation
SE	Standard error
SFC	Solid fat content
SOS	Stearic acid-oleic acid-stearic acid
SRM	Simulated rectal mucus
SS	Supersocolate Special™
SSR	Sum of squares residues
TAG	Triacylglycerol
TGA	Thermogravimetric analysis
T_t	Test formulation
USP	United States Pharmacopoeia
UV	Ultraviolet

W_{ad}	Work of adhesion
%w/v	Percentage weight per volume
%w/w	Percentage weight per weight

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Suppositories are solid dosage forms intended for administration to the human body via insertion into body orifices, mainly the rectum; where it softens, melts or dissolves to release the incorporated medication which then exerts its therapeutic effects locally or systemically. Suppositories can also be administered via the urethra or the vagina (pessaries) (Allen et al., 2008).

Rectal suppositories are usually cylindrical with either one or two tapered ends, shaped like a bullet or a torpedo. Length can be up to 32 mm and it usually weighs about 1–2 g (Allen et al., 2005; Ansel, 1981). The various shapes and sizes of suppositories are shown in Figure 1.1.

Figure 1.1: Shapes and sizes of rectal, vaginal and urethral suppositories (University of North Carolina Eshelman School of Pharmacy, 2015).

Although less common nowadays, suppositories are a relatively old method of administering medications to the human body; dating back to as far as the ancient Egyptian civilization; as evidenced in the Ebers Papyrus scriptures from 1550 BC (Bryan and Smith, 1930).

These days, suppositories are mainly employed as locally-acting laxatives (Table 1.1) to promote defecation or to treat anorectal diseases such as haemorrhoids and ulcerative colitis (Cooper and Gunn, 1987). Nonetheless, there is also a substantial amount of commercial suppository formulations marketed for systemic delivery in Malaysia (Table 1.2). Drugs prescribed as a suppository for systemic treatment include analgesics, antibiotics, tranquilizers and antihistamines.

Table 1.1: The list of commercial suppositories for local action registered with the Drug Control Authority in Malaysia.

Product Name	**Active Ingredient**	**Strengths**
Pentasa	5- aminosalicylic acid, mesalazine	1 g
Salofalk	5- aminosalicylic acid, mesalazine	250, 500 mg
Xyloproct	lignocaine, hydrocortisone acetate, zinc oxide, aluminium subacetate	-
Bisacodyl	bisacodyl	5, 10 mg
Pricolax	bisacodyl	5, 10 mg
Dulcolax	bisacodyl	5, 10 mg
Proctosedyl	hydrocortisone, framycetin sulphate, aesculin, cinchocaine	-
Liproct	hydrocortisone acetate, zinc oxide, lidocaine	-
Doproct	zinc oxide, hydrocortisone acetate, benzocaine	-

Table 1.2 : The list of commercial suppositories for systemic action registered with the Drug Control Authority in Malaysia.

Product Name	Active Ingredient	Strengths
Primperan	metoclopramide hydrochloride	10, 20 mg
Tramadol Stada	tramadol hydrochloride	100 mg
Remedol	paracetamol	125, 250 mg
Arfen	paracetamol	125, 250 mg
Tempol	paracetamol	125, 250 mg
Pritamol	paracetamol	125, 250 mg
Poro	paracetamol	125, 250 mg
Shoren	diclofenac sodium	12.5, 25 mg
Dicloren	diclofenac sodium	12.5 mg
Profenac	diclofenac sodium	12.5 mg
Voltaren	diclofenac sodium	12.5, 25, 50 mg
Almiral	diclofenac sodium	100 mg
Diclogesic	diclofenac sodium	12.5, 100 mg
Voren	diclofenac sodium	12.5, 25, 50, 100 mg
Pritaren	diclofenac sodium	12.5 mg

1.1.1 Advantages of suppositories

Oral drug delivery remains the most common route of drug administration and has the highest rate of patient acceptance. However, the oral route of administering medications may not always be the best option. For example, drugs which undergo extensive first-pass metabolism or pre-systemic degradation such as lidocaine (De Leede et al., 1983), diclofenac sodium (DcNa) (Menasse et al., 1978; Willis et al.,

1979) and salbutamol (Goldstein et al., 1987; Morgan et al., 1986). Conversely, drugs administered to the lower rectum would largely be absorbed into the systemic circulation, thus circumventing the hepatic first-pass metabolism to afford greater bioavailability (Allen et al., 2008; Kokate et al., 2006; Watanabe, 2007). For drugs with unpleasant taste and odour, such as cysteamine (a new treatment for nephropathic cystinosis), suppositories may be an alternative route for drug administration (Buchan, 2011).

Rectal administration of drugs is also particularly useful when the oral route is occluded or disrupted by nausea, vomiting or during acute convulsions (Allen et al., 2005). Nausea and vomiting which limit oral intake of medications are common symptoms in patients undergoing chemotherapy or in palliative care. Hence, alternative routes such as rectal or transdermal drug delivery would be beneficial for these patients (Allen et al., 2005; Davis et al., 2002; Warren, 1996).

Terminally ill and palliative care patients in the outpatient setting would often require frequent administration of multiple analgesics. For these patients, the intravenous (IV) and intramuscular (IM) routes are less practical as they may not always be under the immediate care of qualified health care practitioners.

Clinically, there is also a great deal of unmet needs for alternative routes of administering medications for patients who underwent total gastractomy, ileal resectioning procedures, and patients inserted with nasogastric or nasojejunal tubes where modified release oral formulations are very often rendered less effective. Hence,

non-peroral or transmucosa formulations such as rectal suppositories would be of great value in the outpatient management of such patients.

Furthermore, various recent studies showed that the use of preoperative rectal nonsteroidal anti-inflammatory drug (NSAID) have successfully delayed time before the first request for anaesthesia; reduced the use of supplemental opioids and scored lower on visual analogue scales in both major and minor surgeries in both adults and paediatrics (Bahar et al., 2010; Fayaz et al., 2004).

1.1.2 Patient acceptance and social stigma

A study found that only 18 % of patients favoured suppositories over IM postoperative analgesia (Carroll et al., 1996). Various studies showed that the main reason for rejection of suppository was mainly due to the misconception where patients regard its method of administration as a form of invasion or violation of dignity, leading to subsequent humiliation (Colbert et al., 1998).

Conversely, Vyvyan and Hanafiah (1995) reported that 46 % of the middle aged patients surveyed were receptive towards rectal drug administration; however 98 % of these patients felt the need for discussion prior rectal administration. Meanwhile, Bonner et al. (1996) found that only 15 % of patients aged between 15-91 years old objected to suppository administration under anaesthesia, although 59 % of them preferred to be informed preoperatively. Another study by Dodd et al. (2004) also found high degree of acceptance towards suppositories in women for relief of postnatal pain. Among younger children, Hinton et al. (2007) found that there was considerable acceptance towards the use of suppositories as an alternative route to oral

dosage forms; and that there was also a 69.5 % caregiver acceptance of malarial treatment via rectal route. These were encouraging findings which indicates diminishing stigmatism towards the use of suppositories.

1.1.3 Suppository base

The type of suppository base used depends on the intended release profiles and nature of the active drug. Suppository bases can be classified according to their physical characteristics; fatty bases, water soluble bases and the emulsifying bases (Allen et al., 2005; Cooper and Gunn, 1987).

1.1.3.1 Fatty bases

Suppositories made of fatty (oleaginous) bases must melt upon administration into the rectum before the drug partitions into rectal fluids for absorption across rectal membranes into the systemic circulation (Allen et al., 2008). This group of bases include cocoa butter (CB), palm oil, palm kernel oil (PKO) and cottonseed oil or fat-based glycerine compounds containing high molecular weight (MW) fatty acids such as glyceryl monostearate (Allen et al., 2005). Fatty bases contain very little water and has low tendency of hygroscopicity. Commercialised bases nowadays are usually a combination of two or more fatty bases, for example Wecobee® which is derived from fully hardened palm kernel and cottonseed oils (Stepan Specialty Product, 2014).

1.1.3.1.1 Cocoa butter (CB)

The traditional base, CB or theobroma oil is obtained from the roasted seeds of *theobroma cacao* and presents itself as a yellowish-white solid at room temperature

which melts at 30–36 °C (Allen et al., 2005). CB is desirable due to its melting range, non-irritant nature and miscibility with a wide range of medicaments. However, it exhibits both rancidity and polymorphism on storage. The fatty acid composition of CB is shown in Table 1.3.

It is generally accepted that CB exist in 4 different polymorphs, namely α, β, β' and γ forms although some literatures suggested up to a number of 6 distinct polymorphs (Loisel et al., 1998; Marangoni and McGauley, 2003; van Langevelde et al., 2001; Wille and Lutton, 1966). Each of the polymorphic forms exhibit different melting ranges with β form being most stable. The presence of metastable polymorphs with lower melting points are not conducive for suppositories, especially in warm tropical climates (Allen et al., 2008). Other disadvantages of CB include its inability to contract and detach from suppository moulds on cooling, thus necessitating the lubrication of moulds with liquid paraffin to aid suppository removal (Cooper and Gunn, 1987). There is also substantial batch to batch variation since CB is sourced naturally and the fatty acid content was found to be affected by geographical origin of the CB (Chaiseri and Dimick, 1989; Spangenberg and Dionisi, 2001).

These disadvantages of CB suppository bases prompted the development of newer commercial bases with specific set of properties to overcome formulation difficulties.

Table 1.3 : The composition of the major fatty acids in CB and hydrogenated palm kernel stearin (HPKS).

Fatty acid (carbon no : double bonds)	Weight (%)					
	CB			HPKS		
	Spangenberg and Dionisi (2001)[1]	Lonchampt and Hartel (2004)	Toro-Vazquez et al. (2004)	Rossell (1975)[2]	Siew (2001)	Peyronel and Marangoni (2014)
Lauric (12:0)				49.6	56.6	43.3
Myristic (14:0)	0.09		0.1	30.4	22.0	28.8
Palmitic (16:0)	25.1	26.8	25.8	11.5	7.9	12.6
Stearic (18:0)	37.4	35.6	34.5	2.8	8.6	14.2
Oleic (18:1)	33.0	33.5	34.9	2.4		0.2
Linoleic (18:2)	2.4	3.2	3.0			
Arachidic (20:0)	1.1	0.9	1.0			0.3

[1] Values quoted for sample CB-28, deodorized CB originated from Malaysia.
[2] Values quoted for HPKS (Iodine value =1.8).

1.1.3.1.2 Palm kernel oil (PKO) and hydrogenated palm kernel stearin (HPKS)

Oil palm (*Elaeis guineensis*) is one of the richest vegetable oil plants and is widely used in the food industry. PKO is produced as a by-product via extraction of the residual kernels (Akinoso and Raji, 2011; Pantzaris and Ahmad, 2002; Zhou et al., 2010).

PKO was found to contain 81.67 % of saturated fatty acids; mainly the short-chain fatty acids, such as lauric (C_{12}) and myristic (C_{14}) acid. It has a slip melting point of 27–29 °C and iodine value of 16-20 (Goh, 1994). However, the slip melting point of the PKO can be altered via hydrogenation or blending with other palm oil products (Goh, 1994; Pantzaris and Ahmad, 2002). Further hydrogenation of PKO produces hydrogenated palm kernel stearin (HPKS) with a melting point of 32.5-34.5 °C (Siew and Ng, 2000; Siew, 2001). The common composition for HPKS is shown in Table 1.3.

Recently, Noordin and Chung (2007) developed two new suppository bases using combinations of locally sources hydrogenated PKO, HPKS and hydrogenated palm kernel olein with mixtures of stearic acid and glyceryl monostearate. The authors found that the bioavailability of aspirin administered rectally in these bases were superior to the equivalent dose administered orally.

1.1.3.2 Water soluble and water miscible bases

Unlike fatty bases, water soluble bases disintegrate and dissolve in rectal fluids upon insertion into the human rectum (Allen et al., 2008). Since the rectum has very small amount of fluid, complete vehicle dissolution can be difficult and water will be

attracted from rectal tissues towards the suppository via osmotic effect causing pain to the site of administration. Polyethylene glycol (PEG), poloxamer and glycerinated gelatin are among the common water soluble bases used to produce suppositories.

PEG are polymers made up of ethylene oxide and water, produced in various chain lengths, MW and physical states. It is possible to formulate PEG bases with desired consistency and characteristics by combining different grades of PEGs via fusion process (Allen et al., 2008).

Meanwhile, glycerinated gelatin base could be easily prepared by dissolving 20 % granular gelatin in 70 % of glycerine, added with 10 % of solution or suspension of the desired drug. The resulting base is hygroscopic in nature and could potentially irritate the rectal surface; thus requiring moistening by dipping into water prior to insertion into the rectum (Allen et al., 2008).

Poloxamers on the other hand, are odourless, tasteless, water soluble, block co-polymers which exhibit reverse-thermal gelation properties; they remain liquid at room temperature and undergo phase transition to gel at body temperature (Choi et al., 1998; Keny and Lourenco, 2010). Various studies investigated formulations of thermogelling bioadhesive suppositories using poloxamer in attempts to eliminate discomfort caused by insertion of a conventional solid suppository into the rectum as well as to localise drug absorption within the lower rectum (Barakat, 2009; Choi et al., 1998; Keny and Lourenco, 2010).

1.1.3.3 Emulsifying bases

These include mixtures of oleaginous bases and water miscible materials, disintegrating agents, collagen and natural gums. When formulated as suppositories, it disperses in rectal fluid to form oil-in-water emulsions due to its surface active properties and spreads as a smooth layer over mucous membranes (Allen et al., 2008). As an example, the Witepsol® series composed of triacylglycerols (TAG) of saturated C_{12}-$_{18}$ fatty acids with varied portions of partial glycerides and fatty bases which contain the TAG from palm, palm kernel and coconut oils with self-emulsifying glyceryl monostearate and polyoxyl stearate (Cremer Oleo GmBH & Co. KG).

1.1.4 Ideal base characteristics

Allen et al. (2008) and Cooper and Gunn (1987) summarized that an ideal suppository base should have the following qualities: (1) melt at body temperature or dissolve in body fluids; (2) readily release medicaments; (3) physically and chemically stable; (4) nontoxic, non-irritating and non-sensitizing; (5) compatible with a large variety of drugs; (6) chemically and physiologically inert; (7) contract slightly on cooling; (8) easy to manufacture by fusion, compression and extrusion.

1.1.5 Manufacturing of suppositories

Suppositories can be manufactured via a number of methods, namely hand rolling and shaping, cold-compression and fusion moulding. The method of choice greatly depends on nature of incorporated drug and the scale of manufacturing process as it will not be practical to produce large quantities of suppositories via hand-rolling and shaping method (Allen et al., 2008).

1.1.5.1 Hand rolling

Grated CB and all other required ingredients for the suppository are triturated manually in a mortar to form a plastic-like mass. The mass is then quickly formed into a ball using palms previously cooled in ice water and rolled into a cylinder using a broad bladed spatula over a pill tile. The formed cylindrical mass can then be cut into desired lengths and then shaped as desired by hand (Allen et al., 2008; Ansel, 1981).

1.1.5.2 Cold compression method

In this method, the active drug, suppository base and excipients are blended thoroughly and pulverised to form a uniform blend of mixture which then softens into a paste-like consistency due to the friction of the mixing process (Allen et al., 2008; Ansel, 1981). The paste is then extruded into a mould and compressed for shape setting, the resultant suppositories are then forced out of the mould orifice.

A similar method produces suppositories with uniform circumference by extruding the paste through a perforated plate and cutting the extruded mass into the desired length (Ansel, 1981). This method is suitable for incorporation of thermolabile drugs as the process involves minimal heat exposure. It also enables the incorporation of large amount of drugs that are insoluble in the base as it is unlikely for the insoluble material to settle or separate from the suppository base. Such phenomenon is commonly observed in suppositories produced via fusion moulding method.

1.1.5.3 Fusion moulding method

Fusion moulding method is by far the most commonly employed method to manufacture suppositories as it is compatible with most conventional bases. The

process involves base melting and subsequent incorporation of drug and other excipients into the molten base and pouring the melt into moulds for solidification. The overfilled (excess) base is then scraped off using a warmed spatula to form a smooth flat surface. The nominal capacities of the common moulds are 1, 2, 4 and 8 g (Cooper and Gunn, 1987). This method would require prior calibration of moulds as the densities of bases and drug are different (Allen et al., 2008; Ansel, 1981).

1.2 Human rectum

1.2.1 Anatomy

The human large intestine begins at the colon and extends to the rectum and anal canal at the terminal end (Kokate et al., 2006). The rectum is preceded by sigmoidal colon and ends at anal canal (Watanabe, 2007). The rectum is approximately 15-20 cm in length, with a comparatively small surface area of approximately 200–400 cm^2; while the anal canal is the final 2.5-5 cm of the large intestines leading to the anal verge (Barleben and Mills, 2010).

The rectal wall is made up of three layers; mucosa which composes of several layers of cylindrical epithelial cells; submucosa; tunica muscularis and the visceral peritoneum (Allen et al., 2008). There are three rectal valves in the rectal ampulla - superior, middle and inferior rectal valves. The rectum is usually non-motile and has no villi or microvilli (Allen et al., 2008). When a suppository is administered into the rectum, it either melts or dissolves in the rectal ampulla to release incorporated drug to allow diffusion across the rectal mucosa and subsequent absorption into systemic circulation (Watanabe, 2007).

1.2.2 Rectal mucus

The human rectum contains only 2-3 mL of inert mucus when devoid of faecal matter (Allen et al., 2008). Mucus is a layer of viscous, gel-like secretion by goblet cells which lines all organs of the human body such as the oculo-rhino-otolaryngeal tracts, airways, gastrointestinal tract (GIT), and urogenital tract (Andrews et al., 2009; Bansil and Turner, 2006). It is made up of a mixture of mucin glycoproteins, water, electrolytes, enzymes, bacteria and sloughed epithelial cells (Irons and Robinson, 2003). The bulk of mucus content is approximately 95 % water with 0.5-5 % mucin glycoproteins and lipids, while 0.5-1 % of the contents consist of mineral salts with another 1 % free proteins (Edsman and Hagerstrom, 2005).

This mucus layer functions as a physical barrier to protect the internal environment from pathogens and noxious stimuli; ensures sufficient hydration of the epithelium surface; provides a permeable gel layer for exchange of excretion products, nutrients and gases, and lubricates the epithelium to allow passage of objects (Bansil and Turner, 2006; Irons and Robinson, 2003). Rectal mucus is therefore, the first barrier against diffusion of administered drugs before absorption across the mucosa membranes.

1.2.3 Rectal absorption

The absorptive capacity of human rectum is significantly lesser than upper GIT due to the limited surface area and absence of microvilli compared to small intestines. Drug absorption via rectal mucous membrane is a passive process where only lipophilic, unionised form of drug is absorbed across the membrane (Allen et al., 2008).

The upper rectum is drained by superior hemorrhoidal vein directly into hepatic portal system while the lower rectum is drained by inferior and middle haemorrhoidal veins into the systemic circulation; bypassing first pass metabolic pathways (Kokate et al., 2006). However, the presence of extensive anastomoses may decrease the avoidance of first pass metabolism, although it is generally accepted that at least 50-70 % of active ingredients administered rectally circumvents the first pass effect (Allen et al., 2008; Watanabe, 2007).

1.2.3.1 Factors affecting rectal absorption

Since rectum is not naturally an absorptive organ, the amount of drug absorbed is greatly influenced by various physiologic and physicochemical factors (Table 1.4).

Table 1.4: Summary of factors affecting rectal absorption of drugs (Allen et al., 2008).

Physiological factors	**Physicochemical factors**	**Formulation factors**
colonic contents	nature and form of active drug	microsphere encapsulation of drug
circulation route	physical state of drug	presence of permeation enhancers
rectal fluid pH and buffering capacity	nature of suppository base	bioadhesive properties
volume of rectal fluid	presence of excipients	
motility of rectal wall		

1.2.3.1.1 Physiological factors

There is greater contact between administered suppository and rectal wall for drug absorption to occur when the rectum is empty and devoid of faecal matter, enabling

greater absorption of drug than when it is distended with colonic contents. Likewise, absorption of rectally administered drugs can also be altered by medical conditions such as diarrhoea, colonic obstruction and tissue dehydration (Allen et al., 2005).

Since both upper and lower rectum are drained by superior and inferior haemorrhoidal veins respectively, the position at which the suppository is retained within the rectum could affect systemic bioavailability as drugs absorbed via inferior haemorrhoidal veins avoid first-pass metabolism of the liver (De Leede et al., 1983).

The pH of the rectal fluid is essentially 7.2–7.4, with negligible buffering capacity (Allen et al., 2008; Jantzen et al., 1989; McNeil et al., 1987). Hence, the form of drug incorporated into the suppository would greatly remain chemically unchanged once it is released from the dosage form.

1.2.3.1.2 Physicochemical factors

As with drug absorption across the gastric mucosa, only unionised, undissociated form of drug with sufficient lipophilicity would be able to travel across the bilayer lipid membrane structure of rectal mucosa due to the bilayer lipid membrane structure. However, the drug also has to be sufficiently soluble in rectal fluids to partition away from the lipophilic bases or dissolve from the hydrophilic bases prior to absorption.

The size of drug particles suspended within the suppository base can influence its rate of dissolution in rectal fluids which then affects the rate of absorption. The smaller the size of drug particles, the greater the surface area available for dissolution; thus a faster absorption of drug can be expected (Cooper and Gunn, 1987).

Drugs which are highly soluble in the formulated suppository base tend to exhibit slower drug release than when they are formulated in bases in which they are less soluble (Allen et al., 2008; Ermiş and Tarimci, 1995; Ibrahim et al., 1990; Nair and Bhargava, 1999). This was clearly demonstrated by Nair and Bhargava (1999) where a lipophilic drug fluconazole (log P = 0.44), had highest release rates from the PEG compared to the more lipophilic bases like Suppocire® AP, Witepsol W45 and CB. Therefore, a general rule to optimise drug release would be to formulate hydrophilic drugs in fatty oleaginous suppository bases and lipophilic drugs in hydrophilic bases (Allen et al., 2008, 2005). An alternative method employed to improve drug release is to incorporate surfactants or absorption enhancers into the suppository formulation (Shegokar and Singh, 2010).

Conversely, when sustained release of drug from the suppository is desired, various excipients have been employed to retard drug release from the bases via formation of drug containing micellars using lecithin (Nishihata et al., 1985); solid-reverse-micellar-solutions also using lecithin (Schneeweis and Müller-Goymann, 2000); incorporation of hydrophobic polymers such as hydroxypropyl methylcellulose phthalate HP-55 (Ohnishi et al., 1986) or waxy hydrophobic materials such as aluminium stearate and dioctyl sodium succinate (Ahmed et al., 2000).

1.3 Bioadhesion

Adhesion is the term used to describe the bond produced by interfacial forces when a pressure-sensitive adhesive, either a natural or synthetic polymer, comes into contact with a surface to allow prolonged attachment of the adhesive on the contact surface. Bioadhesion is therefore the interaction which results in the adhesion of the polymer

to a biological surface (Ahuja et al., 1997; Roy and Prabhakar, 2010). Various regions of the body, particularly the GIT is lined by mucosal epithelial which is covered by a layer of continuous mucus (Ahuja et al., 1997; Roy and Prabhakar, 2010).

1.3.1 Theories and mechanisms of bioadhesion

Although exact mechanisms of bioadhesion are not known, it is generally accepted that it involves initial wetting and swelling of the bioadhesive polymer. This is followed by interpenetration between polymer chains and mucosal surface, with subsequent formation of chemical bonds between entangled chains which constitutes the bioadhesion phenomenon. Various theories have been hypothesised to explain the phenomenon.

a) Diffusion theory

This theory postulated that polymer chains of bioadhesive material diffuse into the glycoprotein network and vice versa in a time-dependent manner until there is sufficient interpenetration to form mechanical interlocking and subsequent semi-permanent adhesive bonds, producing a networked structure which results in adhesion (Ahuja et al., 1997; Andrews et al., 2009; Roy and Prabhakar, 2010).

b) Adsorption theory

Adhesion is a result of intermolecular forces acting between atoms in bioadhesive polymer and mucus (Ahuja et al., 1997). Both primary and secondary forces were thought to be involved although adhesion is mainly due to secondary forces such as electrostatic forces, van der Waals forces, hydrogen bonds and hydrophobic interactions (Ahuja et al., 1997; Andrews et al., 2009; Roy and Prabhakar, 2010).

c) Electronic theory

This theory generally stems from the fact that adhesive polymers and mucus typically have different electronic characteristics (Lee et al., 2000). Electron transfer happens when adhesive polymer comes into close contact with the glycoprotein mucus network, forming an electrical double layer. The attractive forces across this double layer results in adhesion (Ahuja et al., 1997; Roy and Prabhakar, 2010).

d) Wetting theory

This theory is applicable for liquids or bioadhesive systems with low viscosity, where the adhesive component would penetrate surface irregularities, harden and anchor itself to the surface (Andrews et al., 2009). If the two adhering surfaces were brought to close contact in the presence of fluid, the fluid could act as an adhesive to attach both surfaces.

e) Fracture theory

The fracture theory evaluates bioadhesion based on difficulty (strain required) to separate two adhering surfaces, which represents strength of the adhesive bond (Ahuja et al., 1997; Andrews et al., 2009).

1.3.2 Factors affecting bioadhesion

The extent of bioadhesion between polymer and mucosa depends largely on polymer properties, environment for bioadhesion and various physiological variables (Table 1.5).

Table 1.5: Factors affecting bioadhesion (Ahuja et al., 1997; Andrews et al., 2009).

Polymer properties	**Physiological factors**
functional group	mucin turnover
degree of hydration	disease states
MW, chain length and degree of cross-linking	pH
polymer concentration	
pH and charge	
swelling	

Polymers containing carboxyl functional groups such as polycarbophils and polyacrylic acid polymers are known to show better bioadhesion at acidic environments whereby bioadhesion decreases with increasing pH. Park and Robinson (1985) found that cross-linked polyacrylic acid has limited bioadhesive properties above pH 6. This was attributed to the ionisation of the carboxyl groups which lead to repulsion between negatively charged carboxylate anions and also a reduction in the formation of hydrogen bonds (Andrews et al., 2009; Park and Robinson, 1985).

Degree of hydration and swelling characteristics of the bioadhesive polymer is also important as swelling relaxes the polymer chains to facilitate interpenetration of the chains. However, excessive swelling has been shown to significantly reduce bioadhesion (Park and Robinson, 1985).

Studies have shown that there is an optimal MW and critical polymer chain length in order to produce bioadhesive interactions (Tobyn et al., 1996, 1995). As polymer MW increases, internal cohesion of the polymer molecule increases, resulting in higher bioadhesion. However, the increase in MW also decreases aqueous dispersibility of

the polymer and hence fewer solubilised carboxylic groups are available for hydrogen bonding (Tobyn et al., 1996). Extensive crosslinking of the polymer also limits flexibility and mobility of the polymer chains, thus impeding penetration and entanglement of the polymer–mucus matrix, resulting in lower bioadhesive forces (Andrews et al., 2009).

Physiologically, mucus turnover is expected to limit residence of bioadhesive dosage forms on the mucosal surface. The rectum has been known to possess relatively low mucus turnover rates as compared to other regions of the GIT and this might be less important in influencing rectal bioadhesion. However, diseased states like inflammatory bowel disease could increase mucus production, or in diarrhoea where there is significantly greater amount of water available (Allen et al., 2008).

1.4 Bioadhesive polymers

Although a large number of polymers exhibit bioadhesive properties, only a selected number of polymers are suitable for pharmaceutical use due to safety considerations. Some of the polymers adapted for pharmaceutical formulations are listed according to their solubility in water and ionic charges in Table 1.6.

Anionic polymers are widely used as bioadhesive polymers due to their strong bioadhesive properties and low toxicity. These polymers are characterised by the presence of carboxyl, hydroxyl, amide or sulphate functional groups which dissociates into negatively charged groups at pH larger than their respective pK_a. Polyacrylic acid polymer such as polycarbophil and carbopol are one of the most popular bioadhesive polymers in pharmaceuticals (Yahagi et al., 2000, 1999).

The most widely used cationic polymer is chitosan; which is derived by hydrolysing the aminoacetyl of chitin from crabs or shrimps. Various studies have reported on the bioadhesivity of chitosan for development of bioadhesive dosage forms, including rectal suppositories (Lehr and Bouwstra, 1992; Tarimci and Ermis, 1997) despite reports that chitosan lacks bioadhesion (Wong et al., 1999a).

Table 1.6: Some of the bioadhesive polymers used in pharmaceutical dosage forms (Irons and Robinson, 2003; Lehr and Bouwstra, 1992).

	Water soluble	**Water insoluble**
Anionic	Alginic Acid Carageenan Sodium carboxymethyl cellulose	Carbopol 934P Polycarbophil Cross-linked polymethacrylic acid
Cationic	Aminodextran	Gelatin Chitosan
Amphiprotic	Carboxymethyl chitosan	
Non-ionic	Polyethylene Glycol Poly(vinylpyrrolidone) Hydroxypropyl cellulose Hydroxypropylmethyl cellulose	Ethyl cellulose

1.4.1 Carbomers

Carbomers are high MW carboxyvinyl polymers which are crosslinked with acrylic acid using either allylsucrose or allyl pentaerythritol (Singla et al., 2000). A typical example of carbomer is Carbopol® (CBP), its chemical structure shown in Figure 1.2a. These polymers are commercially available in various grades depending on MW and structure of the polymeric chain.

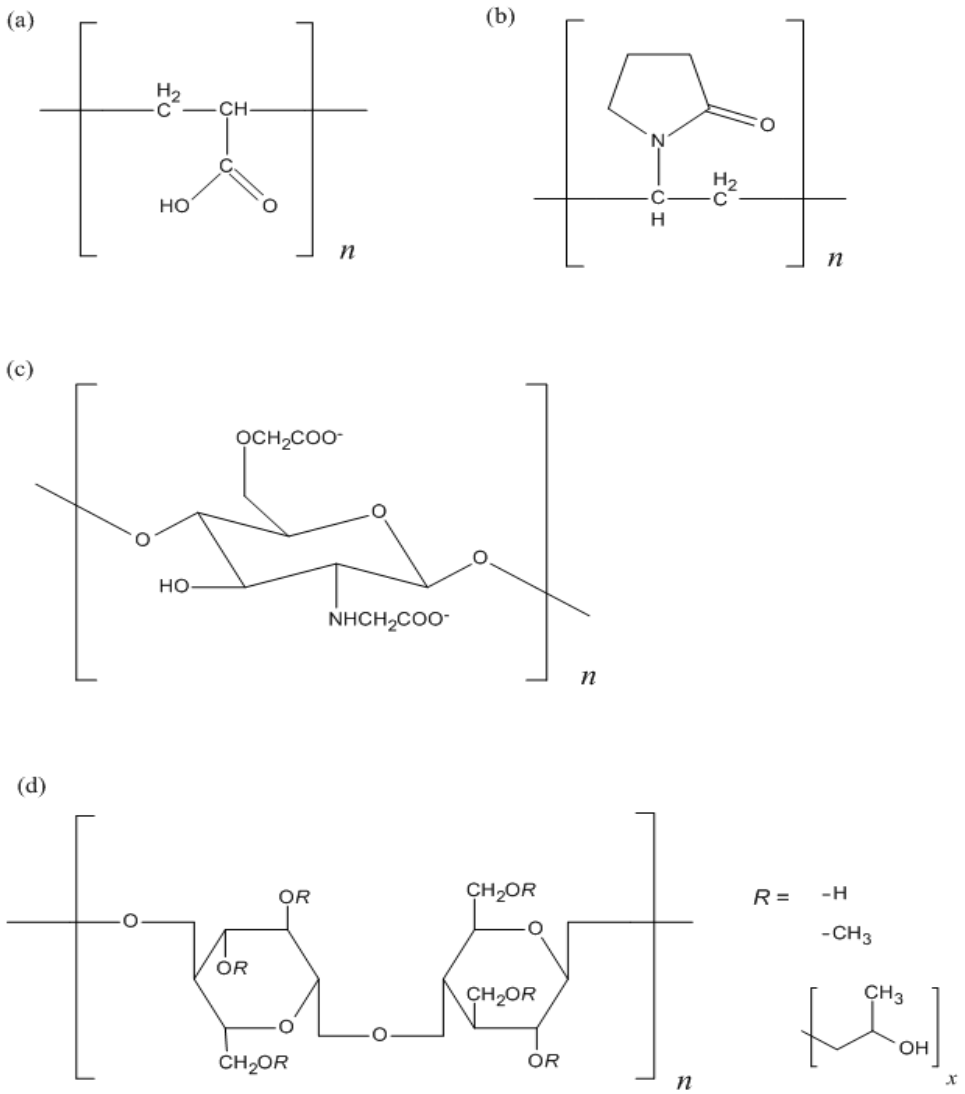


Figure 1.2 : The general chemical structure of bioadhesive polymers used, (a) Carbopol®(CBP); (b) poly(vinylpyrrolidone) (PVP); (c) N,O-carboxymethyl chitosan (CMCTS) and (d) hydroxypropyl methylcellulose (HPMC).

These polymers have been used in the development of various bioadhesive dosage forms such as buccal tablets (Ikinci et al., 2004), transdermal gels (Shin et al., 2005), ophthalmic gels (Edsman et al., 1996), pellets (Gomez-Carracedo et al., 2007), suppositories (Ramadan, 2012; Yahagi et al., 2000, 1999) and more recently, as a bioadhesive conjugate on liposome surfaces (Makhlof et al., 2011; Werle et al., 2010).

CBP is an anionic compound which appears as white, acidic, fluffy, hygroscopic powder. These polymers are insoluble in water and have a pK_a of approximately 6.0 ± 0.5. They are capable of swelling up to 1000 times their volume and 10 times their diameter to produce a gel with pH 4.0–6.0 (Lubrizol Advanced Materials, 2009). At pH above pKa, the carboxylate group on the polymer ionises causing repulsion between the negatively charged polymer backbones, leading to swelling (Lubrizol Advanced Materials, 2009; Singla et al., 2000).

1.4.2 Poly(vinylpyrrolidone)

Poly(vinylpyrrolidone) (PVP) is a synthetic, water soluble neutral polymer produced by free-radical polymerisation of vinylpyrrolidone in water or isopropanolol (Guo et al., 1998). Its general chemical structure is shown in Figure 1.2b. There is no general consensus on the bioadhesive properties of PVP as various studies have reported the absence or limited bioadhesion forces exerted by these polymers. Ivarsson and Wahlgren (2012) and Jones et al. (2004) reported poor or negligible bioadhesion for PVP while other researchers found that formulations containing PVP exhibited considerable bioadhesive strength in the form of coated alginate beads (Suknuntha et al., 2011), tablets (Hamzah et al., 2010), buccal patches (Wong et al., 1999b) and bioadhesive thermogelling rectal gel (Barakat, 2009).

Apart from imparting bioadhesion, PVP was also reported to increase the rate of drug release and sometimes result in a burst of drug release due to its high solubility which promotes pore formation (Islam et al., 2012; Suknuntha et al., 2011).

1.4.3 Chitosan and carboxymethyl chitosan (CMCTS)

Chitosan is derived via partial deacetylation of the natural polysaccharide chitin. Chitosan possesses both hydroxyl (-OH) and amine ($-NH_2$) groups delivering many useful properties such as gel and film forming capacity and bioadhesion (Tungtong et al., 2012). However, the use of chitosan is hampered by its poor solubility at pH > 6.5.

This leads to the incorporation of carboxyl (-COOH) groups to form amphiprotic carboxymethyl chitosan (CMCTS) which permits solubility even at neutral pH, yet retaining good film forming (Mourya et al., 2010) and bioadhesive properties (Shinde et al., 2013) of its parent chitosan. The structure of CMCTS is illustrated in Figure 1.2c. To date, CMCTS has been incorporated into pH sensitive hydrogels (Chen et al., 2004; Vaghani et al., 2012), nanoparticles (Shinde et al., 2013) and transdermal patches (Sarfaraz et al., 2012). However, CMCTS has yet to be formulated into suppositories.

1.4.4 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) is a non-ionic alkyl-hydroxyalkyl cellulose ether derivative containing methoxyl and hydroxypropyl groups (Figure 1.2d). HPMC dissolves in water to form a solution with pH 5.0–8.0 at a concentration of 2 %w/w (Rowe et al., 2009).

HPMC has been incorporated into various drug delivery systems due to its ability to gel on hydration and adhere to both mucin and mucosal surfaces. Due to its nonionic and water soluble nature, possibilities of interaction with other components of the formulation are greatly reduced. It has been incorporated into dosage forms to produce

buccal tablets (Akbari et al., 2010; Wong et al., 1999a), buccal patches (Vishnu et al., 2007) oral bioadhesive tablets (Alladi et al., 2011), bioadhesive vaginal tablets (Bhat and Shivakumar, 2010), bioadhesive transdermal gels (Cho and Choi, 2011) and nasal insert (Bertram and Bodmeier, 2006).

1.5 Bioadhesive suppositories and its advantage

Retention of suppository within the lower rectum via incorporation of bioadhesive polymers enables absorption of the released drug into the lower haemorrhoidal vein which conveniently escapes first-pass degradation. This can be an excellent method to deliver drugs which undergo extensive first-pass metabolism.

Furthermore, the rectum also has relatively low fluid content, thus dissolution of drugs released from suppositories would theoretically be the rate limiting step for drug absorption. Bioadhesive suppositories could potentially overcome this problem by prolonging contact time between molten base and rectal mucosa where absorption takes place. Securing dosage forms at the site of action also builds up a concentration gradient for passive diffusion of drugs across the mucosa (Lehr et al., 1992; Roy and Prabhakar, 2010).

Yahagi et al. (1999) developed double-phased bioadhesive suppositories containing lidocaine using Carbopol 934P and beeswax in Witepsol H15. The group used 10 % carbopol and 20 % beeswax in an attempt to anchor the suppository within the lower rectum. As a result, increased systemic bioavailability of lidocaine from the double phased bioadhesive suppository was observed.

Even when the drug is not extensively degraded during first pass metabolism, suppositories containing bioadhesive polymers have been found to improve systemic bioavailability of ramosetron (an antiemetic) by 2.5 times compared to when the suppository was formulated without bioadhesive component (Yahagi et al., 2000).

1.6 Diclofenac sodium (DcNa)

As one of the more common NSAID, DcNa is prescribed for a myriad of conditions, ranging from long term treatment in patients with rheumatoid arthritis to short term treatment of muscular pains and aches. Recently, there has been great interest in using rectal DcNa for postoperative pain management that could successfully improve pain scores and reduce need for rescue analgesia (Dhawan et al., 2009).

DcNa is scientifically known as sodium (O-((2,6-dichlorophenyl)-amino)-phenyl)-acetate and is a weak acid with pK_a of 4 and a partition coefficient (n-octanol/ aqueous buffer, pH 7.4) of 13.4. The ultraviolet (UV) absorbance of DcNa is detected at a wavelength of 270-276 nm (Palomo et al., 1999).

It has a half-life of 2.3 hours after oral administration and is rapidly excreted from the body. DcNa also precipitates under acidic conditions. Although the drug is fully absorbed into the system, the absolute systemic bioavailability of active drug is only approximately 50 % due to the extensive first-pass metabolism (Willis et al., 1979). Therefore, dosage forms which could effectively avoid the hepatic first pass would technically allow administration of DcNa at lower doses without compromising the clinical responses (Palomo et al., 1999).

A study conducted in healthy volunteers revealed that peak serum levels of unchanged DcNa upon administration of suppository containing 50 mg DcNa occurred within 1 hour, while peak serum levels were only attained after the 2 hours of administration in the subjects given enteric-coated tablets containing equal amount of drug (Reiss et al., 1978). The therapeutic plasma concentration of diclofenac in human was reported to be 0.75–2.0 µg/mL (Winek et al., 2001) although other papers have reported that concentrations as low as 100 ng/mL of plasma diclofenac was effective in initiating analgesic effects (Radermacher et al., 1991). Meanwhile, trough concentrations of Voltaren® SR were found to be 22-25 ng/mL, suggesting that the diclofenac minimum therapeutic concentration might be within the range of 20-50 ng/mL.

To date, DcNa has been commercially marketed as enteric coated tablets, delayed release tablets for oral administration, gel and ointments for topical applications, suppositories for rectal administration and injectable solution for either IM or IV administration. The conventional dose for DcNa orally is 50 mg twice or thrice a day or 75 mg twice a day for adults. The latest addition to the market would be the Voltaren®-XR, an extended release formulation of DcNa for once daily dosing.

1.7 Aims and objectives

Despite being a traditional suppository base, CB has been known to exist in various polymorphic forms, therefore; an alternative base without the extensive polymorphism exhibited by CB is highly desirable. Furthermore, suppositories seem to be an excellent option for delivery of drugs which undergo extensive first pass metabolism because absorption from the lower rectum enters systemic circulation directly.

The aim of this project was therefore to evaluate and characterise bioadhesive DcNa suppositories produced using local HPKS.

Specific aims of the studies in this thesis were:

- to evaluate suitability of HPKS as an alternative to CB as suppository base. HPKS are widely available in Malaysia as a by-product of palm oil at a low cost
- to optimise manufacturing methods for suppositories made with both HPKS and CB using differential scanning calorimetry(DSC)-simulated manufacturing as well as extemporaneous methods
- to physically characterise DcNa suppositories containing bioadhesive polymers made using HPKS in comparison to those made using CB
- to study release of DcNa from various suppository prototypes and the release kinetics involved
- to fabricate and develop novel experimental prototypes for evaluation of bioadhesive properties of suppository prototypes incorporated with bioadhesive polymers (CBP, PVP, HPMC and CMCTS)
- to investigate the feasibility of synthetic membranes as substitute for biological membranes in bioadhesion studies. Synthetic membranes are easily available, standardised and doesn't require prior processing.
- to evaluate physical stability of suppositories under different storage conditions and duration in terms of thermal profile (DSC method), hardness, softening time and drug release

CHAPTER 2

PREFORMULATION STUDIES

2.1 Introduction

The suitability of cocoa butter substitutes (CBS), namely ChocExa (CE) and Supersocolate Special^{TM} (SS) was assessed as part of the preformulation work for development of a fast-acting bioadhesive suppository. Both CE and SS are HPKS derived from kernel of oil palm fruits. CB, a traditional suppository base which was indicated to possess qualities of an ideal suppository base is used for comparison (Ansel, 1981; Kasture et al., 2007).

Although a suppository base is chemically inert and functions only as a carrier for the active drug, properties of the base could affect physicochemical properties of resultant suppositories. Therefore, it is pertinent to characterise the various base properties, namely;

(a) Thermal profile

Two types of thermal analysis; thermogravimetric analysis (TGA) and DSC can be used to study the thermal profile. TGA detects and quantify base decomposition (CB, CE and SS) in terms of mass loss while the DSC shows phase changes encountered by the base upon exposure to temperature range encountered during manufacturing process (Giron, 1986; Schimdt, 2010).

(b) Solid fat content

Solid fat content (SFC) measures the solid-to-liquid ratio in fats over a temperature range. It has traditionally been determined using dilatometry (Walker and Bosin, 1971) and more recently using pulsed nuclear magnetic resonance (*p*-NMR) (Leung et al., 1985) or ultrasonic velocimetry (Singh et al., 2004). An alternative method of SFC

determination is by continuous integration of the DSC thermogram of a sample subjected to heating across a desired temperature range (Menard and Sichina, 2000; Nassu and Gonçalves, 1999).

(c) pH of molten base

Since the rectum is relatively devoid of buffering capacity, pH of the suppository base would determine pH of rectal environment (Ahmad, 2001; Bottger et al., 1989). Certain bases alter pH of rectal environment and this could be potentially problematic if solubility of the drug is highly pH-dependent (Dash and Cudworth, 2001).

(d) Partition coefficient of DcNa in molten base / distilled water

Partition coefficient of DcNa between base and water determines how rapidly and completely it partitions out of suppository in the rectum (Allen et al., 2008). A small partition coefficient (favours aqueous phase) would permit rapid release of DcNa from the bases, supporting the aim of this study to develop fast-release suppositories.

(e) Viscosity of molten base

Viscosity of the molten base during manufacturing affects uniformity of incorporated drug within the dosage form (Allen et al., 2008; Coben and Lieberman, 1986). It is practical that molten base be sufficiently viscous to prevent sedimentation of additives but not too viscous that it makes manufacturing difficult or impedes drug release (Azechi et al., 2000).

Both CE and SS have never been used in the pharmaceutical industry. Thus, proper characterisation of these bases would allow a systematic approach in subsequent

formulation work. This chapter aims to characterise physical properties of HPKS in comparison to CB as conventional suppository base and compares the robustness of the bases towards various manufacturing parameters for subsequent optimisation of CB and HPKS suppository manufacturing methods.

2.2 Materials

CB was purchased from JB Cocoa, Malaysia; while CE and SS were obtained as samples from Lam Soon, Malaysia and Cargill, Malaysia respectively. DcNa was purchased from Shreeji Pharma International, India. Product specifications are included in Appendices 1-3.

2.3 Methods

2.3.1 Characterisation of suppository base

2.3.1.1 Thermal profile

2.3.1.1.1 Thermogravimetric analysis

TGA is conducted by placing 16.0 mg of unprocessed (raw) CB into a 40.0 μL standard aluminium crucibles (Mettler Toledo, USA) sealed with a lid previously pierced with a 50.0 μm hole to relieve vapour entrapment during the thermal cycle. Analysis was carried out using a thermogravimetric analyser connected to a GC10 gas controller system (Mettler Toledo, USA). Suppository bases were scanned at a speed of 1 °C/min across a range of 25 to 70 °C. The flow of nitrogen purge was fixed at 5 mL/min. Experiment was repeated in triplicates.

2.3.1.1.2 Differential scanning calorimetry

DSC was conducted using TA Q2000 (TA Instruments, Delaware, USA) connected to RCS 40 refrigeration system (TA Instruments, Delaware, USA) under nitrogen gas flow of 50 mL/min. Unmanipulated (raw) base samples (CB, CE, SS) of 4-6 mg were crimped into a hermetic Tzero aluminium pan and equilibrated to -10 °C before subjected to heating to 60 °C at a rate of 5 °C/min (first heating). The samples were then cooled to -10 °C at a rate of 5 °C/min before undergoing a second heating to 60 °C. Samples were held isothermally at 60 and -10 °C for 1 minute before cooling and second heating phase respectively. Thermograms were analysed using TA Universal Analysis 2000 software. The melting point is defined as the endothermic peak. Samples were selectively replicated to ensure consistency.

2.3.1.2 Solid fat content (SFC)

Two methods were used to determine SFC in this study; namely the DSC method and *p*-NMR method. The DSC method was modified from Leung et al. (1985) and was conducted using instruments and methods mentioned in Section 2.3.1.1.2. SFC of the raw bases was obtained by continuous integration of the thermogram generated. Experiment was replicated using fresh samples. Determination of SFC using the *p*-NMR was carried out by Malaysian Palm Oil Board (MPOB) using the MPOB p4.9:2004 method.

2.3.1.3 pH of molten base

The pH of molten base (without additives) was determined using methods modified from Dash (2001). 1.0 g of base was placed into a scintillation vial and added with 10.0 mL of distilled water. The vial was then left to shake for 6 hours at 100 rpm in an

isothermic shaker (WiseCube® WIS-20, Wisd Laboratory Instruments) kept at 37.0 ± 0.5 °C. The solution was filtered using a 0.45 µm filter and pH reading of the solution was measured using a calibrated bench top pH meter (Eutech, USA) before and after shaking. Procedures were conducted in triplicates for each base. ANOVA test followed by post hoc Tukey's HSD (Honestly Significant Difference) analysis (SPSS Inc., version 20, USA) was used to analyse the results.

2.3.1.4 Partition coefficient of DcNa in molten base/ distilled water

The partition coefficient of DcNa in the bases were determined using methods modified from studies by Ahmad (2001) and Nayak (2010). 3.0 g of base was weighed into scintillation vials and added with 3.0 mL of 5 %w/v of aqueous DcNa. The vials were sealed and left to shake for 24 hours at 100 rpm in an isothermic shaker (WiseCube® WIS-20, Wisd Laboratory Instruments) kept at 37.0 ± 0.5 °C. The aqueous phase was filtered with 0.45 µm nylon filters and absorbance measured at 276 nm. 3.0 g of base shaken with 3.0 mL of distilled water was used as control. Procedures were conducted in triplicates for each base. ANOVA test followed by post hoc Tukey's HSD (Honestly Significant Difference) analysis (SPSS Inc., version 20, USA) was used to analyse the results.

2.3.1.5 Viscosity of molten base

Rheological properties of only molten bases (without additives) were measured using a LVDV-E rotational viscometer (Brookfield, USA) fitted with DAA cylindrical spindle s87. The small volume sample chamber used was fitted with a water jacket attached to a digital heating circulator unit (Protech, USA) maintained at 37.0 ± 0.5 °C. Molten samples (2 mL) were added into the sample chamber and allowed to

equilibrate to 37.0 °C for 5 minutes. The viscosity measurement in units of centipoise (cp) was obtained at various rotational speeds ranging from 20-100 rpm for torque measurements above 10.0 %. Procedures were conducted in triplicates for each base and expressed as mean ± SD.

2.3.2 Optimisation of manufacturing parameters

Due to similarities in thermal profiles between CE and SS (Section 2.4.1); SS was used as an example of HPKS base in DSC-simulated suppository manufacturing studies (Section 2.3.2.1) and extemporaneous manufacturing of suppositories at various T_{max} (Section 2.3.2.2). The robustness and ease of manufacturing of SS suppositories were compared against conventional suppository base CB.

2.3.2.1 DSC–simulated suppository manufacturing

Simulation of the suppository manufacturing process was conducted using DSC system mentioned in Section 2.3.1.2.2. Unmanipulated (raw) base (CB or SS) of 4-6 mg were subjected to a heat/cool/heat cycle across a predetermined temperature range. The manufacturing parameters which potentially affect polymorphic behaviour of CB and SS were investigated, namely; (a) maximum temperature the base was heated before solidification (T_{max}); (b) heating rate during melting of the base (H_{rate}) and cooling rate for solidification of the molten base to suppositories (C_{rate}). Samples were selectively replicated to ensure consistency.

2.3.2.1.1 Effects of T_{max} on phase behaviour

The first heating prior to a cooling cycle was used to simulate melting of base followed by cooling and solidification in moulds. Independent, freshly prepared

samples were heated to various T_{max} (CB = 34, 35, 36, 37, 38, 39 and 40 °C; SS = 36, 38, 40, 42 and 50 °C) during the first heat cycle. Molten CB was then cooled and equilibrated at 4 °C before the second heating takes place. Second heating cycle was conducted from -10 to 60 °C upon completion of the first heat/cool cycle to identify the polymorphic behaviour of crystallised CB and SS after heating to various T_{max} (during first heat/cool cycle). Heating was conducted at 5 °C/min and cooling at 2 °C/min. Samples were held isothermally for 0.2 minutes in between each heat or cool cycle.

2.3.2.1.2 Effects of H_{rate} and C_{rate} on phase behaviour

To study the effects of H_{rate}, CB samples were heated from -10 to 37 °C while SS samples were heated to 42 °C at various H_{rate} (1, 5 and 10 °C/min) during the first heat cycle and cooled to 4 °C at 2 °C/min. In the C_{rate} studies, CB and SS samples were at 5 °C/min to 37 and 42 °C respectively followed by cooling down to 4 °C at various rates (0.5, 2, 5 and 10 °C/min). The second heat cycle thermograms obtained by heating the samples from -10 to 60 °C at 5 °C/min were used to identify the polymorphic behaviour of crystallised CB and SS after being subjected to different H_{rate} and C_{rate} in the first heat/cool cycle.

2.3.2.2 Extemporaneous manufacturing of suppositories at various T_{max}

Two types of suppositories were produced in this section for evaluation, base-only suppositories (blank) and suppositories containing the model drug DcNa. The suppositories were prepared by fusion method. Base (CB or SS) was heated over a water bath (Julabo, Germany) to various T_{max} temperatures (CB heated to 32, 34, 36, 37 and 42 ± 0.5 °C; SS to 36, 40, 42 and 50 ± 0.5 °C).

In DcNa-containing suppositories, DcNa was added into the molten base with gradual stirring before pouring into the 1.0 g steel suppository mould cavities to cool at controlled room temperature of 22 ± 1.5 °C; relative humidity (RH) 63 ± 3 % until solidification of molten base. The produced suppositories each contained 50 mg DcNa. Blanks were moulded without DcNa into the molten. Suppositories were then placed into the refrigerator (3.5 ± 1.5 °C) for additional 10 minutes. The excess base overfilling the mould cavities were scrapped off using a warm spatula to produce suppositories with a smooth, flat end. The produced blank suppositories were scraped and 4-6 mg of sample was crimped into a hermetic aluminium pan and heated in the DSC from -10 to 60 °C at 5 °C/min. The ease of manufacturing suppositories at various molten temperatures as well as the quality of the manufactured suppositories was assessed. Quality of manufactured suppositories was evaluated based on the presence or absence of cracks, bubbles or discoloration and well as smoothness to touch.

2.3.2.3 Determination of displacement value (DV)

Blank suppositories (CB, CE and SS) were produced using calibrated six-cavity steel suppository moulds (each cavity 1.0 g) and the suppositories produced were weighed. The same moulds were used to manufacture suppositories containing 10 %w/w DcNa. The medicated suppositories were weighed.

Displacement value (DV) determination was repeated in triplicates for each base and calculated using Equation 2.1 as stated by Mollel (2006) and Vidras et al. (1982) :

Equation 2.1 $$F = \frac{XB}{100\,(A - B) - XB}$$

Where,

F = displacement value

X = percentage of additive used (DcNa)

B = weight of suppositories containing X % additive

A = weight of blank suppositories made without any additives

2.4 Results and discussion

2.4.1 Characterisation of suppository base

Based on the thermogravimetric data generated, the bases appear to be stable over the temperature range of 25 to 70 °C. Weight loss of the base was minimal and insignificant, thus it was concluded that decomposition did not occur across the range of temperature that they are exposed to during manufacturing process.

Due to the large variability in naming of polymorphic forms in published literature, the polymorphic forms for CB and both HPKS (CE and SS) encountered in this thesis will be referred to as described in Tables 2.1-2.2 respectively. Designation of polymorphic forms was based on their respective melting points in comparison to previous published literature.

Table 2.1 : Reported polymorphic forms of CB and the variability in their nomenclature. Table includes nomenclature used to describe CB polymorphs in this thesis.

Melting range (°C)	Von Vaeck (1960)	Wille and Lutton (1966)	Lovegren et al. (1976)	Van Malssen et al. (1999)	Allen et al. (2008)	Beckett (2008)	Nomenclature in thesis (CB)
-5 to +5				γ			1 (Not observed)
12 to 15			VI			I	2
16 to 20	γ	I	V	α	γ		
21 to 24	α	II	IV	‡β’	α	II	
25 to 27		§III	III			III	3A
27 to 30	β’	IV	II		β’	IV	3B
29 to 34	β	V	I	β		V	4A
34 to 36		VI			β	VI	4B

‡ Van Malssen et al. (1999) suggested that β’ exist as a range rather than two distinct forms, recorded by Beckett (2008) and Wille and Lutton (1966) as forms III and IV; and forms II, III and IV by Lovegren et al. (1976).

§ Form III was subsequently found to be a mixture of different proportions of forms II and IV, as confirmed in studies by Aronhime et al. (1988).

Table 2.2 : The reported polymorphic forms of lauric fats, PKO blends, HPKS and the variability in their nomenclature. Table includes nomenclature used to describe CE and SS polymorphs in this thesis.

	Lauric fats	PKO blend**	HPKS			
Melting range (°C)	Anihouvi et al. (2013)	Noordin and Chung (2009)	Siew (2001)	Peyronel and Marangoni (2014)	Rossell (1975)	Nomenclature in thesis (CE and SS) ††
-30 to -10	α					Not observed
20 to 28		Form I				Not observed
28 to 32	β’	Form II				β'_2
33 to 34		Form III	β’	β’		β'_1
35 to 37					‡‡β’	β

** Custom blend composed of HPKS: PKS: virgin PKO at ratio of 5:2:3.

†† Iodine value of 0.4 in CE and 0.29 in SS.

‡‡ Melting point quoted for β’ forms from HPKS are based on four different degrees of hydrogenation and iodine value (IV) of 8.3, 4.4, 2.5 and 0.4.

The first heating thermograms in Figure 2.1 (solid lines) depicted melting profiles of the unmanipulated (raw) bases; while the second melt thermogram (dash lines) obtained from reheating the bases after first heating and cooling showed tolerability of the bases to subsequent solidification after heating to high temperatures.

At least three distinct polymorphs were observed in the unmanipulated CB based on the first heating thermogram (Figure 2.1a, solid line). They were form 2 (11.9 °C), form 3A (22.1 °C), form 3B (28.8 °C) and form 4B (34.7 °C). Form 1 was not observed throughout this study due to its metastable nature which resulted in its spontaneous crystallisation into form 2 polymorph (Dewettinck and Foubert, 2004). The CB stock used in this study has been stored for about 3 years at 3.5 ± 1.5 °C, stabilisation of the form 4A into form 4B polymorph over the storage duration resulted in the existence of form 4B as the major component in unmanipulated CB.

Upon cooling after the first heating, CB existed mainly in form 2 which exhibits an endotherm peak at 20.3 °C (melting of form 2) and to a much lesser extent, form 3B which melted at 26.6 °C (Figure 2.1a, dashed line). The presence of these polymorphic forms is unfavourable, as the suppositories would theoretically remain in the liquid state at room temperatures, causing sedimentation and separation of the active drug or additives from the base.

Previous studies by Siew (2001) suggested that HPKS predominantly crystallises into β' polymorph which has a melting endotherm at 32.5 °C followed by a shoulder at 35–38 °C. The author also observed an exothermic event at 22-25 °C suggestive of a transition from lower melting polymorphs to the more stable β'.

Both the HPKS used in this study were found to have lesser polymorphic forms compared to CB. In the first heating cycle, CE (Figure 2.1b, solid line) yielded three distinct melting endotherms; β'_2 presented as a small peak at 27.0 °C, a larger endotherm at 34.1 °C by the β'_1 form and a less visible shoulder around 37.1 °C attributed to the presence of β form. This was consistent with the HPKS melting points reported by Chapman et al. (1971). Upon cooling, molten CE crystallised into the β'_2 form which exhibited a single melting endotherm at 31.7 °C, as observed in the second heating thermogram (Figure 2.1b, dash line).

Similarly, SS displayed three different forms; the β'_2 form (27.9 °C), β'_1 form (35.7 °C), and β form (present as a shoulder around 38.4 °C) (Figure 2.1c, solid line). Upon first heating and cooling, SS crystallised into β'_2 which melts at 31.9 °C (Figure 2.1, dash line). Both CE and SS existed in polymorphic forms with melting points at approximately 32 °C after the first heat/cool cycle, despite a slight shift in the endotherm peak to a lower temperature compared to the first heating. This reflected the robustness of HPKS over CB towards temperature manipulation.

All three bases had melting points close to the human body temperature, which is an essential characteristic of a suppository base.

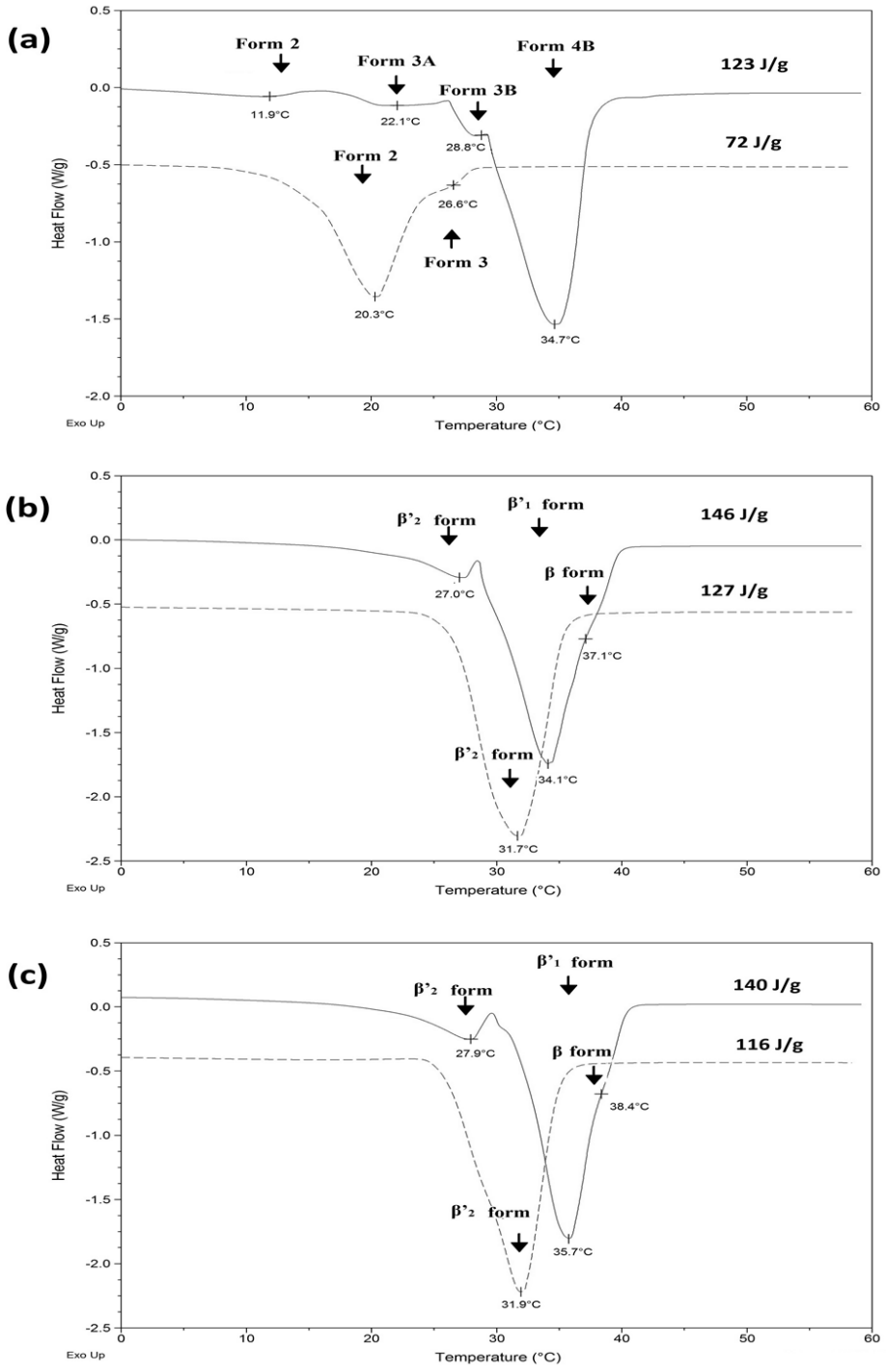


Figure 2.1: DSC thermograms showing the first and second heating profiles of unadulterated (raw) bases (a) CB; (b) CE and (c) SS. Solid lines and dash lines indicate the first and second heating respectively. Thermograms were offset for clarity. Enthalpy of fusion was included on far right of each thermogram.

Figure 2.2a shows the SFC of the three bases determined using the DSC method. The melting integral of a thermogram corresponds to the proportion of melted fat at a particular temperature (Marquez et al., 2013). The SFC curves (Figure 2.2a) of all three bases (CB, CE, SS) were similar and have very steep slopes between 32-38 °C.

Comparatively, the SFC generated using the *p*-NMR (Figure 2.2b) showed that curves for both CE and SS were almost identical but were skewed to the right of the CB curve. This was due to the experimental methods where CB samples have been heated up to 80 °C to clear thermal memory before analysis, thus the SFC curve is likely to correspond to the less stable polymorphic form of CB. The DSC method allows continuous measurement of a single sample across the entire range of temperature and allows the measurement of unadulterated (raw) samples; hence preferred for studying the processing behaviour of the base during suppository manufacturing. In general, both the HPKS (CE and SS) remained solid until higher temperatures than CB before undergoing a similar sharp melting over a narrow range of temperature.

The SFC of fats can be used as a prognostic indicator of essential properties; for example, the SFC below 25 °C provides an indication of hardness of the particular fat, while the SFC between 25-30 °C reflects its resistance to heating (Torbica et al., 2005). Meanwhile, the steepness of the slope at 35 ± 2 °C is more relevant in suppositories as it reflects how rapidly the suppository melts at human body temperature.

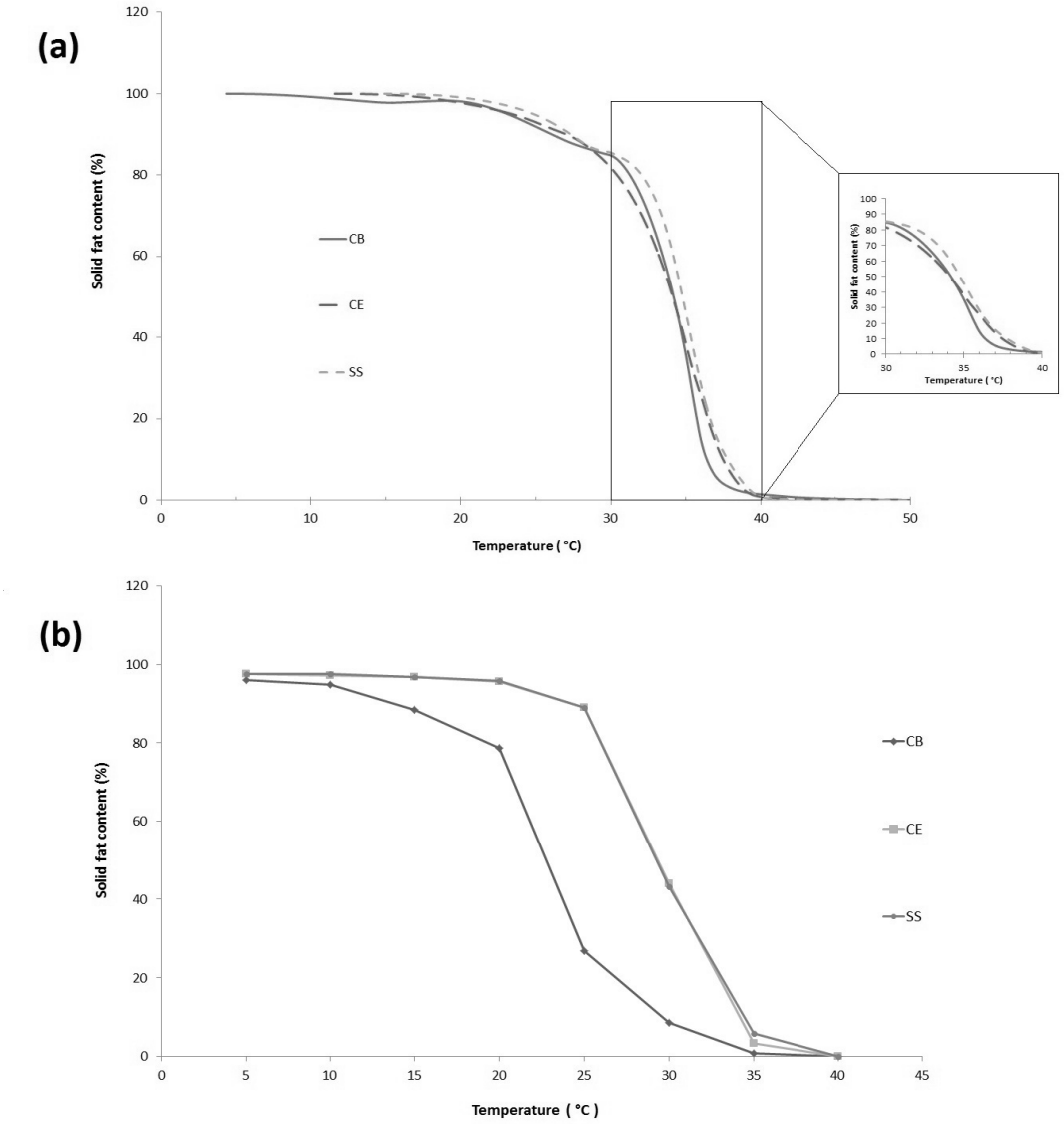


Figure 2.2 : SFC (%) of unadulterated (raw) suppository bases (CB, CE and SS) determined using (a) DSC method and (b) *p*-NMR method (MPOB p4.9 : 2004).

Meanwhile, none of the three bases significantly altered pH of distilled water upon melting ($p > 0.05$). The model drug DcNa have been reported to have a solubility of 0.0036 mg/mL in pH 4.5 acetate buffer to 0.036 mg/mL at pH 5.3 where a modest increment of pH by 0.8 lead to a 10-fold increase in solubility of DcNa (Chuasuwan et al., 2009). Therefore, any changes in pH brought about by melting of bases could

potentially affect the solubility of DcNa and its release from the bases; considering the lack of buffering capacity in the rectal cavity.

An important attribute of a suppository base is its ability to release the incorporated drug completely upon melting. The melting profile, viscosity, partition ratio and solubility of drug in surrounding aqueous environment impacts the ease of drug release. If a drug has higher solubility in the fatty base over the aqueous phase (rectum environment) then it may be incompletely released from the suppositories or released slowly (Allen et al., 2008). DcNa was found to preferentially partition into the aqueous phase over oily phase in all the three studied bases. This suggested that all the three bases release DcNa rapidly, although the partition ratio (base/water) of DcNa in CB (0.245 ± 0.007) was significantly higher ($p < 0.05$) than both CE (0.031 ± 0.003) and SS (0.037 ± 0.008).

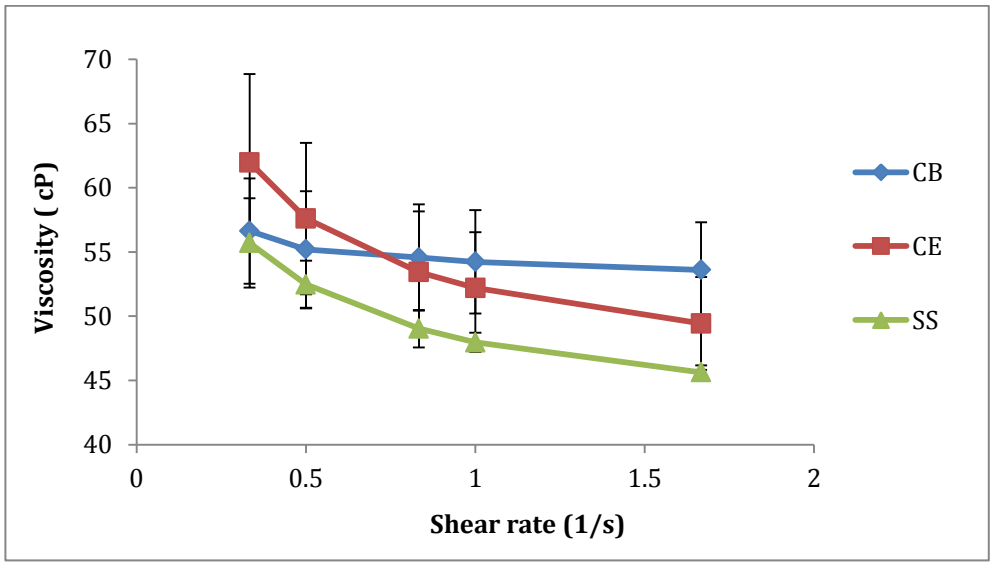


Figure 2.3 : The viscosity of the bases (CB, CE and SS) under different shear rates maintained at 37 ± 0.5 ºC. Mean ± SD, n=3.

Figure 2.3 showed that the viscosities of all three bases decrease with increasing shear rate. CE generally had similar viscosity to CB over shear rate of 20–100 rpm at 37 ºC while SS appeared less viscous than both CE and CB. CE and SS are mainly composed of shorter-chained lauric acid (C_{12}) while the longer stearic acid (C_{18}) predominates in CB (Allen et al., 1999; Gros and Feuge, 1952). A molten base with lower viscosity may offer better spreadability in the rectum upon melting, thus increasing surface area for drug release, but at the same time extremely low viscosities risk sedimentation of drug particles during manufacturing. The lower viscosities in CE and SS in this situation were compensated by a steep SFC profile (Figure 2.2), as rapid solidification reduces risk of drug sedimentation.

2.4.2 Optimisation of suppository manufacturing methods

The first heating in the heat/cool/heat cycle in the DSC-simulated manufacturing of suppositories was conducted to simulate heating up of the base; this is then followed by cooling of the molten to produce solid suppositories. Finally, the second heating was used to determine the melting profile of the suppositories produced.

The small endothermic peak observed in the 10-20 °C regions (Figures 2.4a–d, inset) in CB samples was attributed to melting of metastable form 2. The entailing exothermic peak observed at the range of 15–25 °C corresponded to direct solid-solid transition of the metastable form 2 to form 3A (Dewettinck and Foubert, 2004), which intensity increases proportionally to increasing amounts of form 2 present. CB molten heated to a T_{max} between 34-37 °C (Figures 2.4a–d) crystallised mainly into form 2 as well as a mixture of form 3A and 3B. Similar to previous studies, the experiments carried out in this study using DSC were unable to distinguish between the form 2 to

form 3 transitions and the direct crystallisation of form 3 from the melt (Toro-Vazquez et al., 2004). The presence of a small peak at approximately 36 °C (Figures 2.4a–c, asterisk) simply implied the incomplete melting of form 4B in the initial raw base before cooling down rather than as a result of the thermal manipulation imposed. The size of form 4B peak decreased as the T_{max} was increased (Figures 2.4a–c) and completely diminished when T_{max} was above 37 °C (Figures 2.4d–g).

When CB was heated to T_{max} > 37 °C, the main polymorphic form produced was form 2 with small amounts of form 3A which was present as a shoulder at 25–28 °C (Figures 2.4e-g, solid arrow). Complete removal of crystal 'memory' followed by continuous cooling to 4 °C at 2 °C/min resulted in formation of unstable form 2 polymorph. The small amounts of form 3A observed could be a result of the spontaneous transition from any form 2 polymorph produced (Dewettinck and Foubert, 2004). This highlighted the narrow temperature derangement tolerated by CB during manufacturing before the base preferentially crystallises into form 2 polymorph.

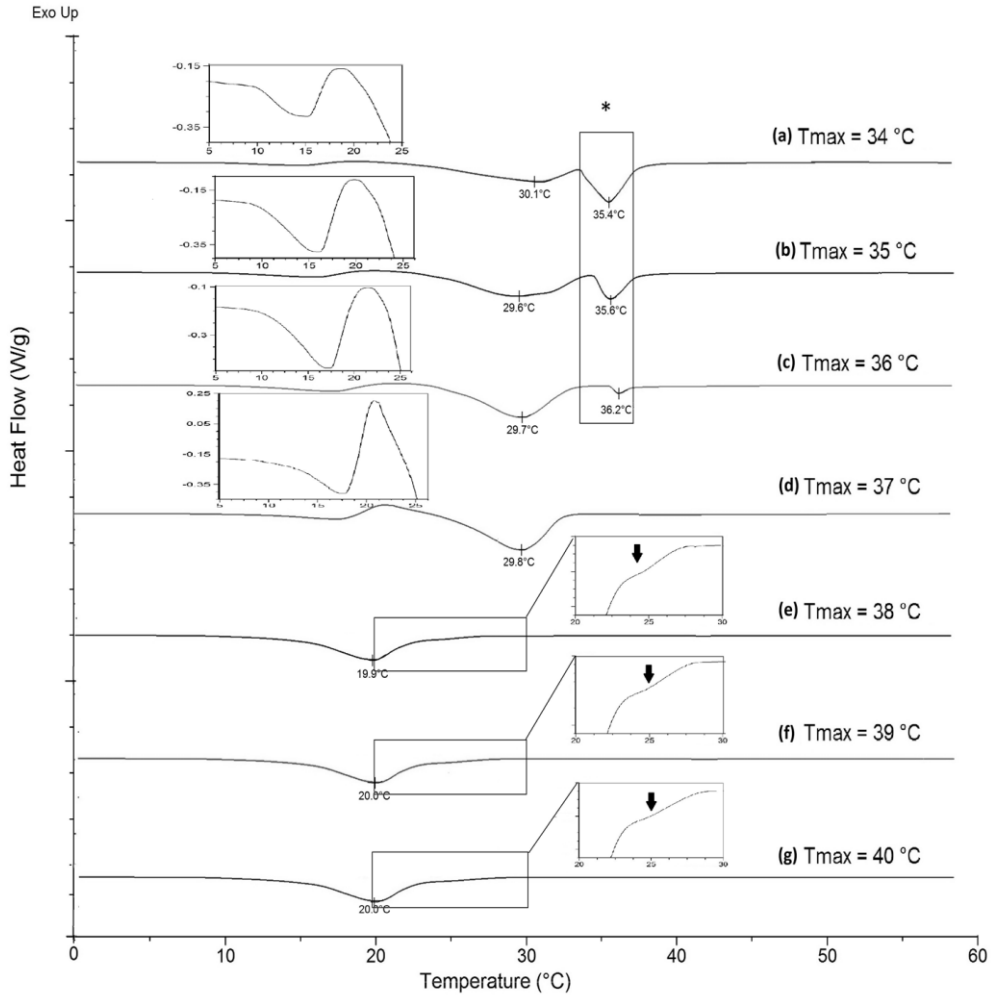


Figure 2.4 : Thermograms of the second heating cycle of CB samples. CB was subjected to various T_{max} (5 °C/min) before being cooled at 2 °C/min to 4 °C. Subtle peaks are enlarged in the respective figure inset. Arrow denotes small amounts of form 3A polymorph present as a shoulder. Asterisk denotes residual form 4B polymorph from first heating.

Figures 2.5a–f showed that SS base heated to between 36 and 50 °C recrystallised into a single polymorphic form, the stable β'_2 form (31-32 °C). This was consistent with previous studies which reported the preferential crystallisation of lauric fats into the β'_2 polymorph (Anihouvi et al., 2013; Ehlers, 2012; Rossell, 1975; Timms, 1984).

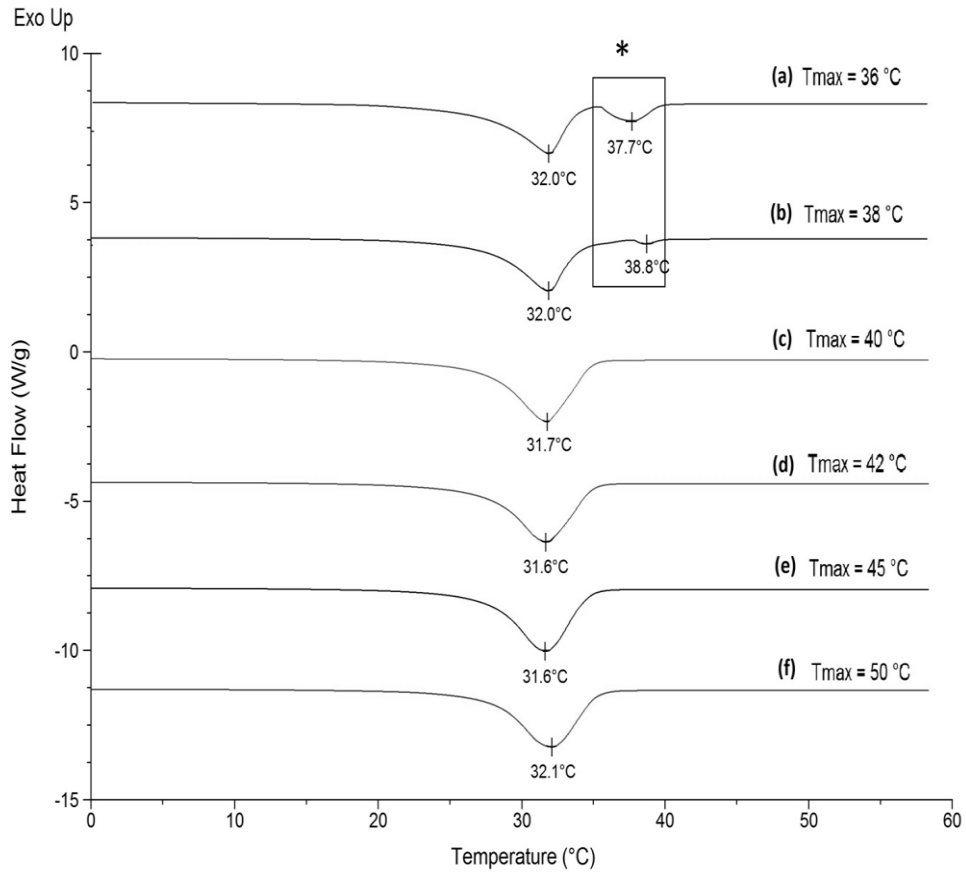


Figure 2.5 : Thermograms of the second heating cycle of SS samples. SS was subjected to various T_{max} heated at 5 °C/min before being cooled at 2 °C/min to 4 °C. Asterisk denotes residual β polymorph from first heating.

Presence of a small peak around 37-39 °C (Figures 2.5a–b, asterisk) was thought to be due to incomplete melting of β form in the base before the cooling cycle. SS has a wide range of T_{max} tolerance of at least 10 °C (Figures 2.5c–f), a contrasting difference to CB. This simplifies manufacturing of suppositories using SS as it required less stringent temperature control. As long as SS is completely molten (>38 °C), it would solidify into a stable β'_2 polymorph. This trend was observed for T_{max} up to 50 °C.

On the contrary, there was a narrow range of T_{max} between 34-38 °C which the CB base has to conform to during manufacturing. CB has to be heated up to the temperature at which it completely melts, yet ensuring that it does not overheat to produce form 1 (α form) or require excessively long time for solidification. Unlike CB, crystallisation of SS was not affected by T_{max} during manufacturing using DSC-simulated methods across the T_{max} range of 40-50 °C.

Figures 2.6a–c showed the crystallisation of molten CB previously heated to 37 °C upon cooling at 2, 0.5 and 0.2 °C/min. CB molten cooled at 2 °C/min has three distinct crystallisation exotherms at 21.9, 18.3 and 14.4 °C respectively (Figure 2.6a). At cooling rate of 0.5 °C/min, there were two crystallisation exotherms at 21.5 and 10.5 °C (Figure 2.6b), while cooling rate of 0.2 °C/min resulted in a single crystallisation exotherm at 23.3 °C (Figure 2.6c). This was translated into the different polymorphic forms as characterised by distinct endothermic peaks observed upon reheating (Figures 2.6d-f). At the cooling rates investigated, CB crystallised mainly into form 4A (Figures 2.6d-f, open arrow). CB cooled at 2 °C/min contained considerable amounts of form 2 (Figures 2.6d-e, solid arrow) which diminished as C_{rate} was lowered. Presence of form 2 is supported by the appearance of an exothermic peak around 18–20 °C (Figures 2.6a-b, asterisk) which corresponded to its transition to 3A polymorph. Melting of this 3A polymorph appeared as a faint shoulder around 22–26 °C (Figures 2.6a-b; hash). This exothermic peak at 18–20 °C was absent when the C_{rate} was reduced to 0.5 and 0.2 °C/min (Figure 2.6f).

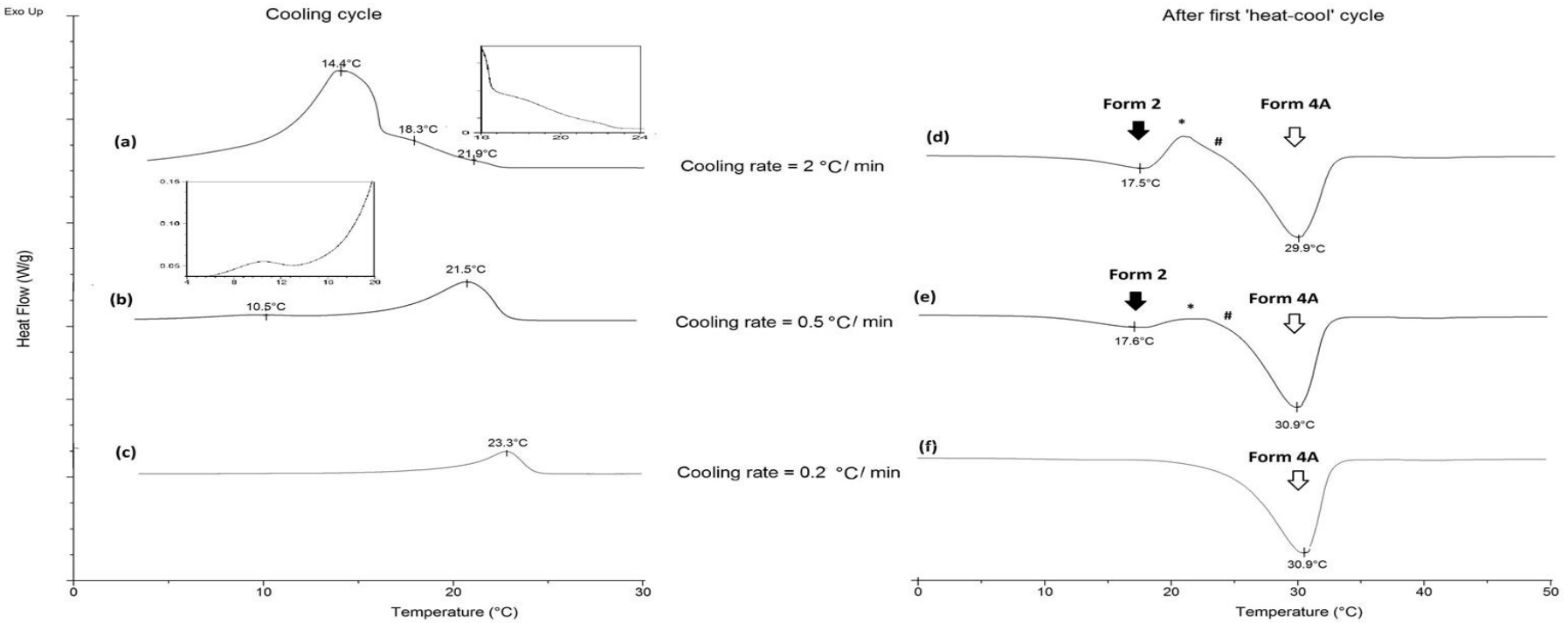


Figure 2.6 : Thermograms of the cooling cycle of CB samples at (a) 2 °C/min, (b) 0.5 °C/min and (c) 0.2 °C/min after undergoing first heating to T_{max} of 37 °C. Thermograms of the second heating cycle are shown in the corresponding thermograms (d–f). First heating was conducted at 5 °C/min and subjected to solidification at various cooling rates. Subtle peaks are shown in figure insets. Solid arrows denote presence of form 2 while open arrows denote form 4A. Asterisks denote exothermic event while hashes indicate presence of form 3A.

Even when CB was heated to 38 °C, it may still be possible for the molten to crystallise into form 3B polymorph (Figure 2.7d, asterisk) with a melting point at 30.2 °C via slow cooling at 0.5 °C/min, albeit in mixture with substantial amounts of metastable form 2 (Figure 2.7d, solid arrow). However, the formation of 3B polymorph decreased when CB was heated to 39 °C (Figure 2.7e, asterisk) and completely diminished with T_{max} of 40 °C (Figure 2.7f). In CB heated to 40 °C, slow cooling at 0.5 °C/min produced only form 2 polymorphs (Figure 2.7f, solid arrow). CB heated to 38-40 °C crystallised mainly into form 2 (Figures 2.7a-c, solid arrow) with small amounts of 3A polymorphs (Figures 2.7a-c, open arrow) upon cooling at 2 °C/min. The difference in C_{rate} greatly affects crystallisation and polymorphic form of molten CB. Rapid cooling resulted in an unstable diffuse crystalline phase made up of low energy polymorphs, while slow cooling allows more time for the TAG chains to pack into a lamellae to form a stable, 3-D structure (Metin and Hartel, 2005).

In molten SS however, the C_{rate} had less influence on the resultant polymorphic forms. SS consistently underwent a single step crystallisation process (Figures 2.8(i)a-d) into the stable β' polymorph (mixture of β'_1 and β'_2) with a melting endotherm at 31-33 °C (Figures 2.8(ii)a-d, solid arrow); regardless of the C_{rate} between 0.2-5 °C/min. This robustness observed in SS is favourable especially for the manufacturing of suppositories as it eliminates requirement for rate controlled cooling and prevents variability of polymorph formation in the final product.

The H_{rate} on the other hand, was found to not affect the polymorphic forms produced in both CB and SS between 1–10 °C/min (results not shown).

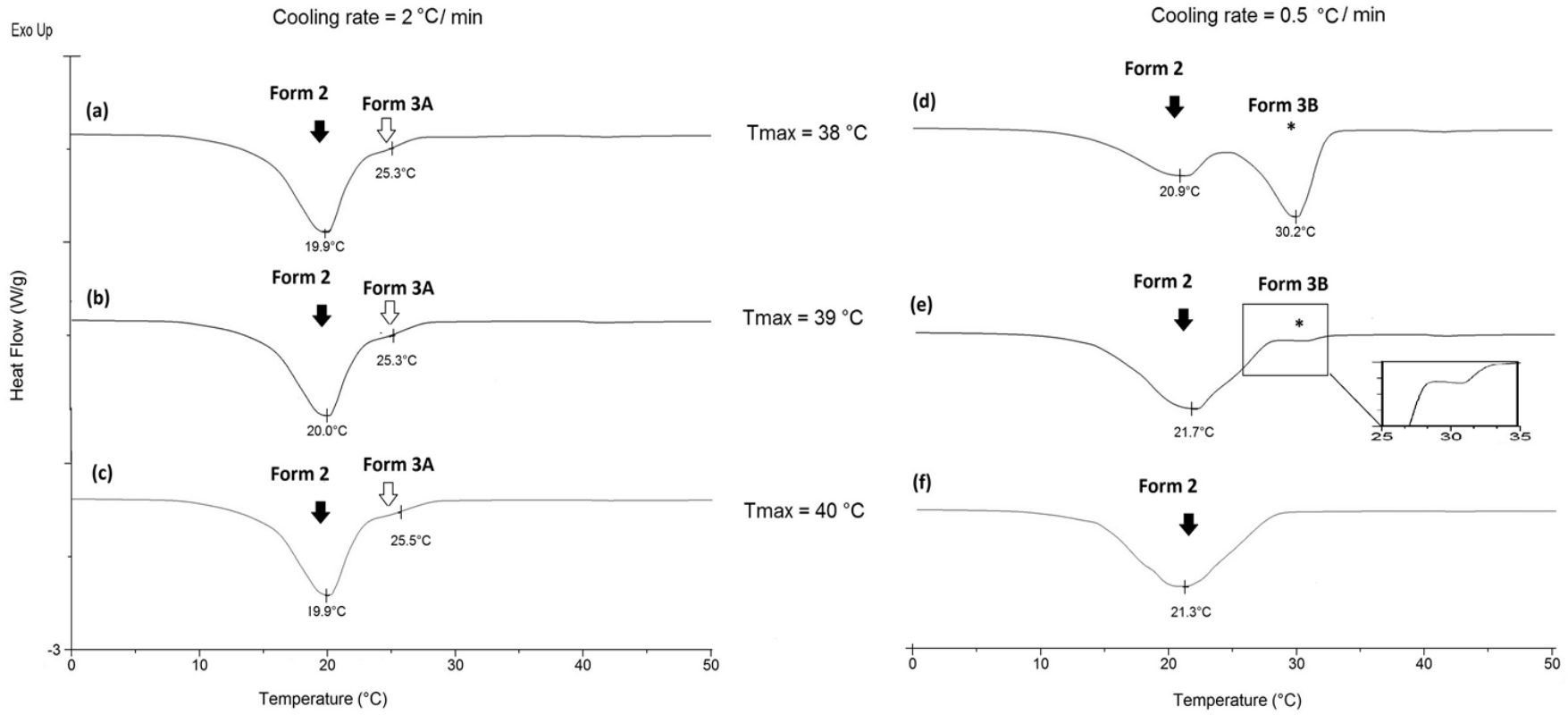


Figure 2.7 : Thermograms of the second heating cycle of the CB. First heating was conducted to different T_{max} 38, 39 and 40 °C (5 °C/min) and subjected to solidification at either cooling rate of 2 °C/min (a–c) or 0.5 °C/min (d–f). Smaller peaks are shown in the figure insets. Solid arrows denote presence of form 2; while open arrows denote form 3A. Asterisks denote form 3B.

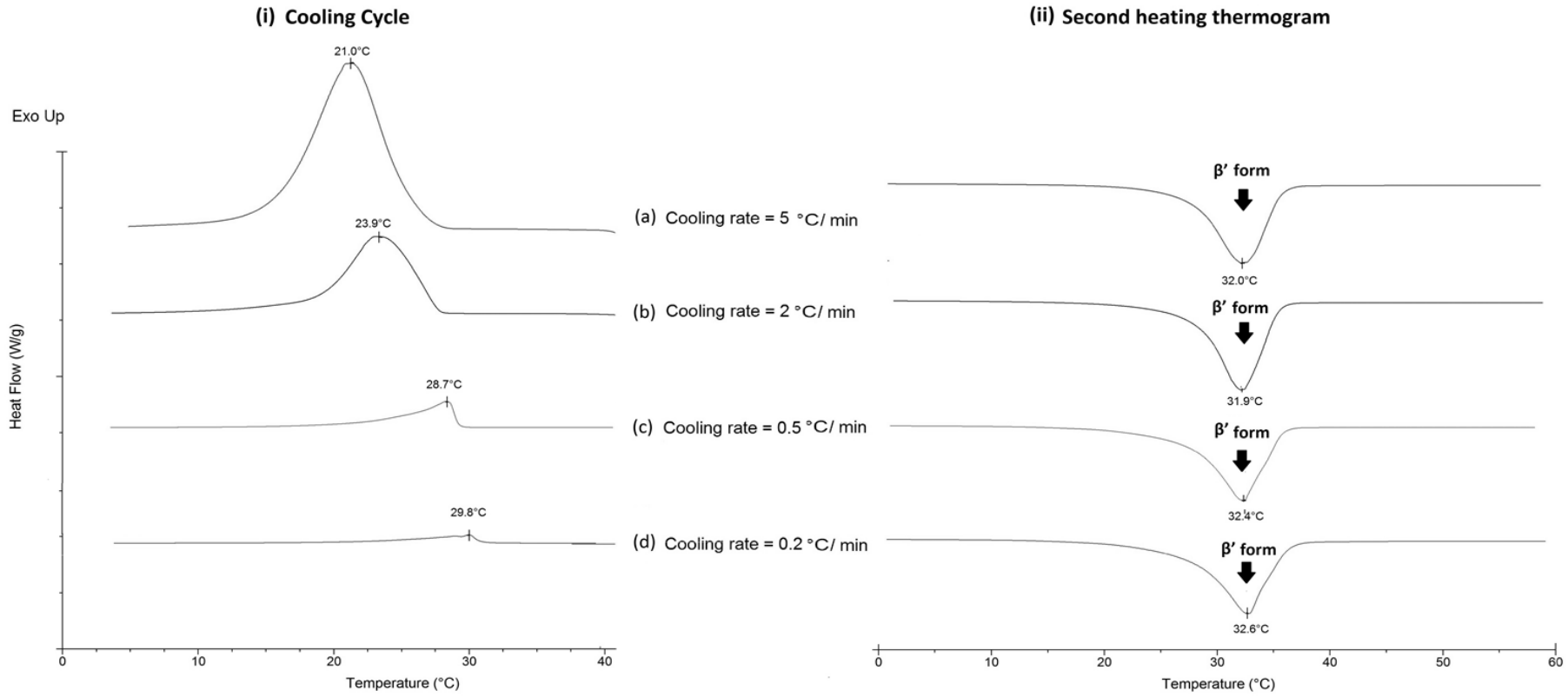


Figure 2.8 : Thermograms of (i) cooling cycle and (ii) second heating thermogram after first 'heat–cool' cycle of SS samples. The SS samples were subjected to cooling rate of (a) 5 °C/min, (b) 2 °C/min, (c) 0.5 °C/min and (d) 0.2 °C/min after first heating to T_{max} of 42 °C. Both first and second heating cycles were conducted at rate of 5 °C/min. Solid arrows denote presence of β' form (mixture of β'_1 and β'_2).

The C_{rate} of molten base in extemporaneously prepared suppositories was ~1 °C/min, projected based on the time required for molten base temperature to decrease by 10 °C during actual manufacturing.

All extemporaneously produced suppositories from CB molten at various T_{max} resulted in similar profiles, with the exception of molten CB at T_{max} of 32 °C where an additional small endothermic peak at 36.5 °C (Figure 2.9a, asterisk) was a result of the incomplete melting of the unmanipulated (raw) CB base during manufacturing. This was consistent with observations in DSC simulated studies (Figures 2.4a–c). The main endothermic peak at 32.4 ± 0.3 °C indicated that CB suppositories existed mainly in the stable form 4A (Figures 2.9a-e, open arrow), with a shoulder at 25–29 °C attributed to the presence of one or more form 3 polymorphs (Figures 2.9a-e, solid arrow). The proportion of form 3 polymorphs present increased with increasing T_{max}. A small peak at 10–20 °C was observed which corresponded to very small amounts of form 2 polymorph (Figures 2.9a-e, inset marked with hash).

This is an interesting finding since even molten which has been heated to 40 °C congealed to produce suppositories in the stable form 4A polymorphs (Figures 2.9a-e), rather than into form 2 polymorphs as observed in the DSC-simulated studies and as suggested by various literatures (Collett, 1990). It was deduced from these observations, that even when molten CB has been heated above 36 °C, it was still possible to manufacture suppositories in the stable form 4A as long as the molten CB has been allowed to congeal over longer periods of time (ie: >55 mins) at temperatures between 21.5–23.5 °C. If the same molten had been rapidly cooled in the refrigerator (simulated by the DSC cooling), it would have congealed under

refrigeration into form 2 polymorph which reverts to a molten once removed from the refrigerator, owing to its low melting point.

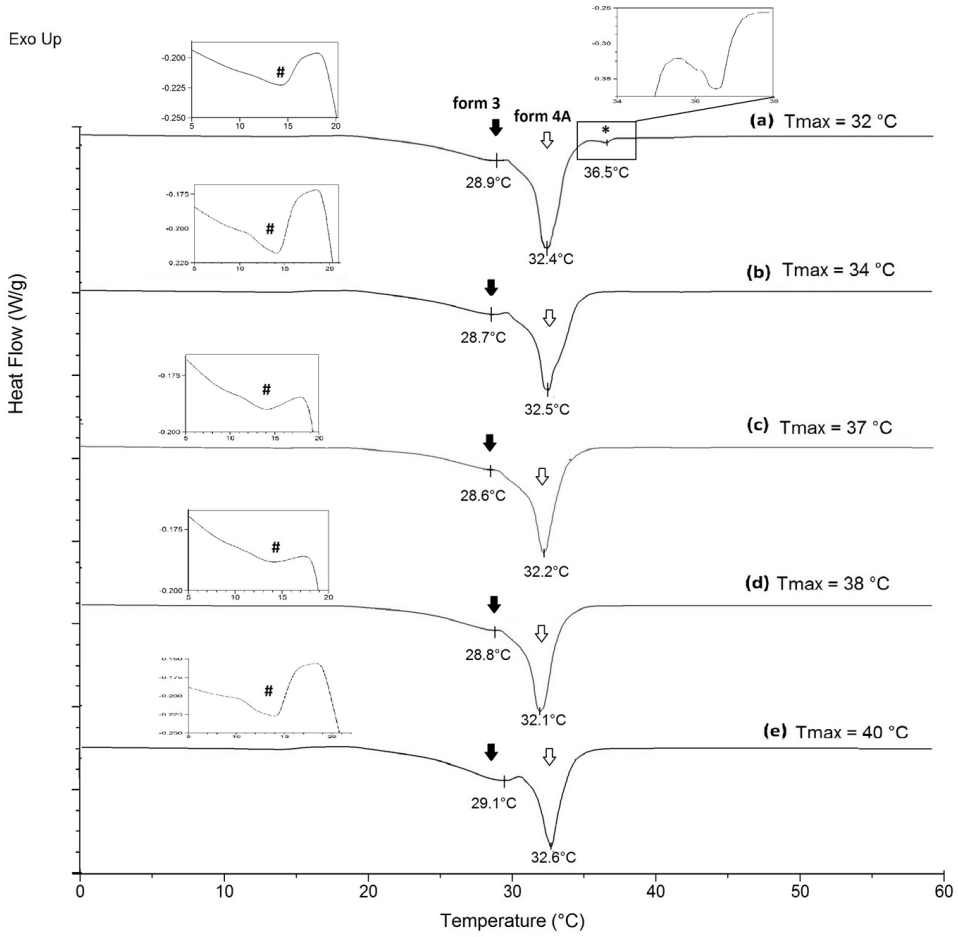


Figure 2.9 : Thermograms of extemporaneously produced CB suppositories manufactured from molten heated to various T_{max} followed by solidification at regulated lab conditions of 22.5 ± 1 °C; RH 63 ± 3 %. Smaller peaks are shown in the enlarged insets. Hashes indicate the form 2 polymorph, while solid and open arrows denote the presence of form 3 and form 4A polymorphs respectively. Asterisk shows the unmelted residual (raw) base.

On the other hand, molten SS appeared white and creamy when heated up to 39 °C. At 40 °C, SS becomes a colourless liquid. The SS suppositories produced from

molten at T_{max} of 40 to 50 °C consisted mainly of the stable β'_1 polymorph (Figures 2.10a-d, open arrow) with a melting point of 34.3 ± 0.1 °C, together with a small fraction of β'_2 (Figure 2.10, solid arrow), evident as a small shoulder at 29.4 ± 0.5 °C. The small peak present at 40.1 °C (Figure 2.10a, asterisk) was a result of residual unmanipulated (raw) base due to incomplete melting.

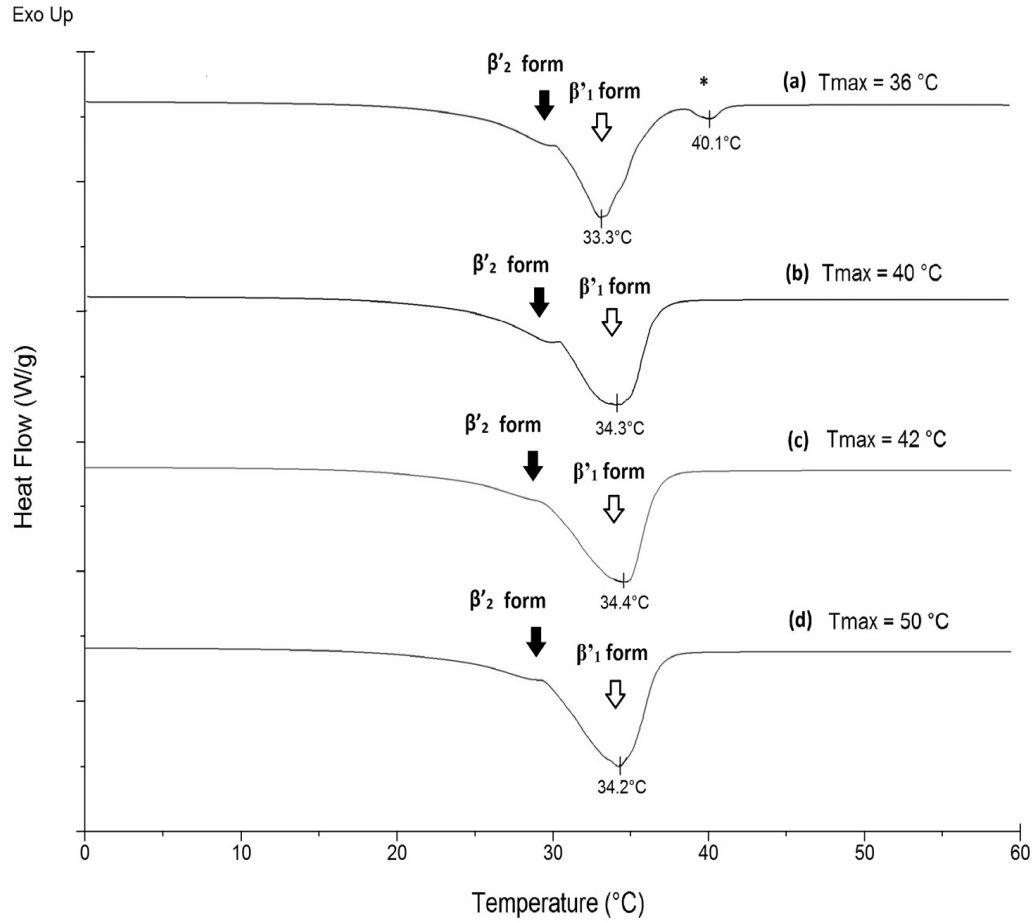


Figure 2.10 : Thermograms of extemporaneously produced SS suppositories manufactured from molten heated to various T_{max} followed by solidification at regulated lab conditions of 22.5 C ± 1 °C; RH 63 ± 3 %. Solid arrow denotes the presence of small amounts of β'_2 form, while open arrow denotes β'_1 form. Asterisk shows the unmelted residual base.

A summary of the qualitative assessment can be found in Table 2.3. It was found that molten CB heated to 32 °C and below was too thick for DcNa incorporation and made pouring into moulds difficult. On the other hand, molten CB heated to 38 °C and 42 °C resulted in solidification time more than 60 minutes and sedimentation of DcNa particles to the bottom of the suppositories was observed. Incorporation of DcNa into SS on the other hand, was easy in molten heated to temperatures above 36 °C.

The observed differences for CB and SS between both methods (DSC-simulated method and extemporaneous manufacturing) were mainly due to differences in experimental setup. Extemporaneously compounded suppositories were manufactured in a lab with temperatures regulated at 22 ± 1.5 °C; RH 63 ± 3 %, while the samples in DSC-simulated studies were crimped into a pan and cooled at a fixed rate. Moreover, the molten bases in extemporaneous manufactured suppositories were subjected to stirring (shear) which facilitated rearrangement of the fatty acid chains into a more stable arrangement (Dhonsi and Stapley, 2006).

The effects of temperature and shear on crystallisation and polymorphic forms produced has been well demonstrated by various groups (Dhonsi and Stapley, 2006; Macmillan and Roberts, 2002; Sato, 2001). In the presence of shearing in CB, form 4A polymorphs were produced as a result of transformation from the precursor form 3A (Dhonsi and Stapley, 2006; Sato, 2001). While in the absence of shearing, only the form 3 variants (both form 3A and 3B) were observed, with form 3A polymorph again acting as a precursor for the formation of more stable form 3B (Sato, 2001).

Table 2.3 : Qualitative assessment of CB and SS molten properties, ease of manufacturing process and visual appearance of compounded suppositories subjected to various $T_{max,}$ as determined by lab-scale extemporaneous manufacturing process. [§§]

T_{max} (°C)		**CB**					**SS**			
		30	**32**	**34**	**37**	**40**	**36**	**40**	**42**	**50**
Molten properties	Colour	Pale yellow	Milky yellow	Yellow	Dark yellow	Dark yellow	White	Colourless	Colourless	Colourless
	Bubbles	-	-	-	+	+	-	-	-	-
	Turbidity	++	+	-	-	-	++	-	-	-
	Viscosity	++	+	-	-	-	++	-	-	-
Ease of manufacturing	Drug incorporation	Unable to mould	Difficult (thick base)	Easy	Easy	Difficult (sediment)	Difficult (thick base)	Easy	Easy	Easy
	Pouring into mould		Difficult (viscous)	Easy	Easy	Easy	Difficult (viscous)	Easy	Easy	Easy
	Leaking from mould		-	-	+	++	-	-	-	-
	Time to solidification (min)		15	18	54	65	5	7	10	18
Produced suppositories	Colour		Yellow	Yellow	Yellow	Yellow	White	White	White	White
	Smoothness		Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Cracks		-	-	-	-	-	-	-	-
	Airholes (Bubbles)		+	-	-	-	+	+	+	+

[§§] '+' implies presence of a particular observation; '++' implies presence of the observation with greater intensity; while '-' implies the absence of the observation.

Similarly, extemporaneously manufactured SS suppositories comprised of the higher melting point β'_1 polymorph (33-34 °C) in the presence of shear (from stirring of molten) rather than the lower melting point β'_2 polymorph observed in the DSC-simulated studies.

Based on both DSC-simulated and extemporaneous manufacturing of suppositories, T_{max} of 34 and 42 °C were used in production of CB and SS suppositories respectively for subsequent analysis considering their practical solidification time.

The DV of a compound is the weight of medicament or additive which displaces 1.0 g of the base in which they are to be formulated in (Allen et al., 2008). These values are crucial to determine the exact quantities of excipients, active drug and bases needed to formulate each suppository as the moulds used during manufacturing are of a fixed volume (Allen et al., 2008; Babar et al., 1999; Vidras et al., 1982).

DV of the same drug could be different for different bases due to difference in density, hence complicating the manufacturing process and resulting in inaccurate dosage strength. This study found that the DV of DcNa was similar among the three bases (CB = 1.40 ± 0.06; CE = 1.40 ± 0.02 and; SS = 1.48 ± 0.07). CE and SS may be a good substitute or equivalent for CB as a suppository base in existing formulations using CB.

2.5 Conclusion

Both the HPKS used in this study, CE and SS were found to be comparable to CB in terms of thermal profile, SFC, pH, viscosity and DV.

This chapter also found that CB has rigid processing parameters during manufacturing. When manufactured extemporaneously, it is crucial that the CB molten base is (1) maintained between 34 ± 0.5 °C during heating and (2) allowed to cool slowly to between 20–24 °C to produce suppositories with the desirable form 4A polymorph (3) molten CB should not be placed into a refrigerator before solidification is complete as it will result in rapid crystallisation into the form 2 polymorph. These restrictions however, were not relevant in HPKS where crystallisation was independent of the heating and cooling process during manufacturing. Stirring of molten (shear) promotes the formation of higher melting point polymorphic forms in both bases. HPKS is a suitable alternative suppository base due to its superior manufacturing flexibility compared to CB.

Based on the findings in this chapter, the two HPKS investigated (CE and SS) appear to be suitable candidates as an alternative lipophilic base for the development of DcNa suppositories.

CHAPTER 3

FORMULATION AND ASSESSMENT OF SUPPOSITORY DOSAGE FORM

3.1 Introduction

3.1.1 Routine analysis for manufactured suppositories

Analytical assessments of manufactured pharmaceutical products are routinely conducted to ensure quality standards are met. Following manufacture, suppositories are physically inspected for its appearance; examined for weight uniformity, melting point, viscosity, active drug content, resistance to mechanical fracture (hardness) and softening time (Coben and Lieberman, 1986; De Blaey and Tukker, 1996).

Visual appearance of suppositories can sometimes be a good indication of the homogeneity of suspended ingredients as well as suitability of the manufacturing parameters (Allen et al., 2008; De Blaey and Tukker, 1996). Meanwhile, significant weight variation in a batch of suppositories could be an indication of either inhomogeneity of additives or the presence of a cavity which ultimately affects content uniformity of dosage forms. Proper standardisation of manufacturing parameters would minimise these variations (Mollel, 2006).

Melting point of a lipophilic suppository is crucial for drug release upon insertion into the rectum. A depressed melting point leads to melting and damage of the suppositories during handling while those with melting point above body temperature will not melt completely. Various methods have previously been used to study melting point of suppositories, including the DSC (Kauss et al., 2013; Mollel, 2006; Yahagi et al., 1999), capillary method (Ahmad, 2001; Hammouda et al., 1993; Pugunes and Ugandar, 2013) and water bath method (Shegokar and Singh, 2010; Soremekun et al., 2012). The DSC method offers a more precise measurement as it records actual phase changes of the base across the temperature range tested.

Viscosity of melted suppositories would affect spreading and release of medication *in situ* (Azechi et al., 2000). Thicker molten may hinder drug release and limit spreading while excessively watery molten could leak from the rectum. Suppository viscosities have been measured using viscometers of the Ubbelohde type (Yahagi et al., 2000), cone and plate type (Takatori et al., 2004) and spindle viscometer (Reanmongkol et al., 2011; Victoria and David, 2003).

Determination of the active drug (DcNa) content of suppositories is crucial to demonstrate dose-to-dose consistency. Method of active drug quantification ranges from the more conventional solvent or aqueous extraction of the drug followed by subsequent spectrophotometric analysis (Shegokar and Singh, 2010; Swamy et al., 2012; Zawar and Bhandari, 2012); by way of DSC micro quantification method (Noordin and Chung, 2004) or by using the partial least squares treatment of the FT-Raman spectra (Szostak and Mazurek, 2013).

Additives are known to affect hardness of suppositories (Coben and Lieberman, 1986; Güneri et al., 2004; Kosior, 2001; Shegokar and Singh, 2010). Although there is no standard method to evaluate hardness of suppositories, measurements have been done using suppository hardness tester type SBT by Erweka (Babar et al., 1999; Ghorab et al., 2011; Güneri et al., 2004; Hanaee et al., 2004; Shegokar and Singh, 2010), bench top tablet hardness tester (El-Majri and Sharma, 2010; Kurosawa et al., 1985; Oribe et al., 1995), hand-held Monsanto tablet hardness tester (Noman and Kadi, 2011; Saleem et al., 2008; Varshney Himanshu and Tanwar, 2009), modified rheometer with tooth press stick B (Ramadan, 2012; Yahagi et al., 1999), and texture analyser (Gugulothu et al., 2010).

Softening time is especially important for lipophilic suppositories. A long softening time may result in premature expulsion from the rectum before drug absorption can occur, yet suppositories which soften too soon are easily damaged during handling prior to administration. The European Pharmacopoeia (2010) specified an apparatus in Section 2.9.22 which consisted of a glass tube immersed in a temperature controlled water bath for the measurement of time elapsed for lipophilic suppositories to soften permitting penetration of rod probe. Apart from that, softening time can also be determined using the liquefaction and softening time apparatus (Moghimipour et al., 2009) and U-tube submerged in a water-bath (Varshney Himanshu and Tanwar, 2009).

This chapter aims to manufacture bioadhesive suppositories containing DcNa (model drug) and four different types of bioadhesive polymers using the fusion method. The polymers used were the anionic polymer CBP grade 974P NF; amphiprotic polymer CMCTS and non-ionic polymers PVP grade K30 and HPMC grade 2910. The manufactured suppositories were then subjected to routine analysis in terms of physical inspection, weight variation, melting point, DcNa content, viscosity, hardness and softening time in order to ensure the quality standards are met.

3.2 Materials

The base and model drug used have been described in Section 2.2. CBP 974P NF was obtained as a sample from Drex-chem (M) Sdn. Bhd., Malaysia; HPMC 2910 was purchased from Newstar Chem Enterprise, China; PVP K30 was acquired from Brightchem Sdn Bhd., Malaysia; while CMCTS was procured from China Eastar Co. Ltd., China. Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen

phosphate (K_2HPO_4) and potassium bromide were purchased from Nacalai Tesque Inc., Kyoto, Japan. Product specifications are included in Appendices 4-7.

3.3 Methods

3.3.1 Manufacturing of DcNa suppositories containing bioadhesive polymers

Methods of suppository manufacturing have been optimised in Section 2.4. The DV for bioadhesive polymers were determined using methods described in Section 2.3.3.3 for all three bases used.

The actual amount of suppository base required for a specific batch of suppositories was calculated using Equation 3.1. The base required was accurately weighed into a ceramic evaporating dish using a lab analytical balance (Sartorius, Germany) and heated over a water bath (Julabo, Germany) to 34.0 °C for CB and 42.0 °C for both the HPKS (CE and SS). Accurately weighed DcNa and bioadhesive polymers were evenly mixed into the molten with gradual stirring before pouring into the 1.0 g steel suppository mould cavities. Suppositories were allowed to congeal at room temperature for 30 minutes before the overfilled excess was scraped-off using a heated spatula. The suppositories produced were stored at 3.5 ± 1.5 °C, RH 29 ± 3 %; and used within 2 weeks of manufacturing. Five suppository formulations were produced for the subsequent analyses; base-only suppositories (blank), suppositories containing only 50 mg DcNa (DcNa only) and suppositories containing 50 mg DcNa added with 1, 2 or 5 %w/w bioadhesive polymers (CBP, PVP, HPMC, CMCTS). The resultant suppositories were 25 mm in length and 8 mm in diameter at the barrel end.

Equation 3.1 $$B = (N \; x \; F) - \left[\left(\frac{A_1}{D_1}\right) + \left(\frac{A_2}{D_2}\right)\right]$$

Where,

B = amount of base required

N = number of suppositories to prepare

F = fill weight of mould cavities obtained from mould calibration

A_1 = required amount of additive-1

D_1 = displacement values of additive-1 in the base used

A_2 = required amount of additive-2

D_2 = displacement values of additive-2 in the base used

3.3.2 Dosage form analysis

3.3.2.1 Visual inspection

Physical appearances of the external surface of 10 randomly selected suppositories (n=10) from each formulation were examined for odour, shape integrity, colour uniformity, and manufacturing defects.

3.3.2.2 Weight variation

The mass of 10 randomly selected suppositories (n=10) from each formulation were individually weighed. Data obtained was presented as mean weight ± SD.

The target weight (g) of individual suppositories for each formulation was calculated as a comparison to the actual weight of the suppositories produced, using the equation:

Equation 3.2 $$Target\ weight\ = \frac{WB_N + WD_N + WP_N}{N}$$

Where,

WB_N = weight of base used (CB, CE or SS) to produce one batch

WD_N = weight of active drug used (DcNa) to produce one batch

WP_N = weight of bioadhesive polymer (CBP, HPMC, PVP, CMCTS) for one batch

N = number of suppositories to produce in one batch

3.3.2.3 Melting point

The melting point of all the formulations was determined using DSC parameters described for determination of melting point of the bases in Section 2.3.1.1. The endothermic peak minimum on the DSC thermogram was identified as melting point of the formulation tested.

3.3.2.4 Determination of DcNa content

The suppositories were stirred in 200 mL phosphate buffer (pH 7.4) solution on a Variomag® Telesystem magnetic stirrer plate (Thermoelectron Corp, WI, USA) immersed in a 37.0 °C water bath (Fisher Scientific, PA, USA) for 3 hours. The content of DcNa released from each suppository was quantified spectrophotometrically. Only formulations incorporated with 5 %w/w bioadhesive polymers (CBP, PVP, HPMC and CMCTS) were examined for content uniformity. Suppositories containing 50 mg DcNa without bioadhesive polymer was examined for comparison purposes.

3.3.2.5 Viscosity

Viscosity measurements of the molten suppositories were carried out in the same manner as Section 2.3.1.5. The measurement obtained at shear rate of 50 rpm was used for comparison purposes between the base-only suppositories, suppositories containing only DcNa and suppositories containing DcNa and 1–5 %w/w bioadhesive polymers. The experiment was carried out in triplicates and the results were expressed as mean ± SD.

3.3.2.6 Hardness

Hardness of the formulated suppositories was measured using Dr. Schleuniger 8M Tablet Hardness Tester (Pharmatron, USA). The hardness tests were carried out at room temperature. Hardness measurements in units of Newton (N) were taken both at the cylindrical circumference (Figure 3.1a) as well as the pointed tip (Figure 3.1b) of the suppositories. Measurements were repeated with six independent samples (n=6) for each batches tested for both the forces required to break the barrel and tip of the suppository.

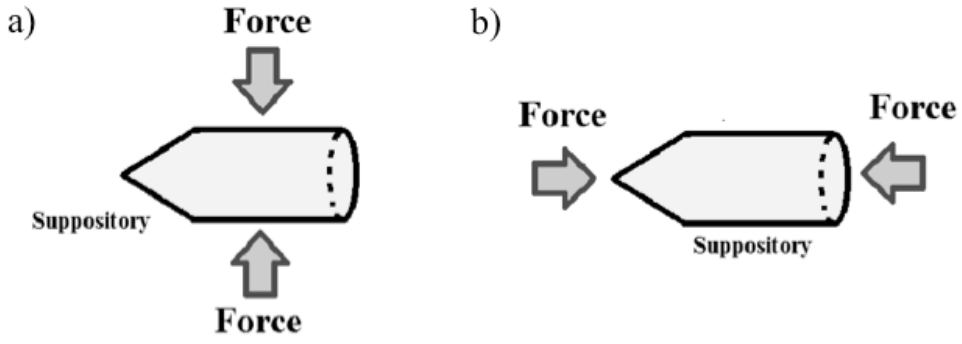


Figure 3.1: Direction of force exerted at (a) cylindrical circumference (barrel) (b) pointed tip of suppository.

3.3.2.7 Softening time

The softening time of suppositories were measured using a modified apparatus (Figure 3.2) adapted from the Erweka Suppository Penetration Tester PM 30 (Erweka, Germany) and SPT-6 Penetration Tester (Pharmatest, USA).

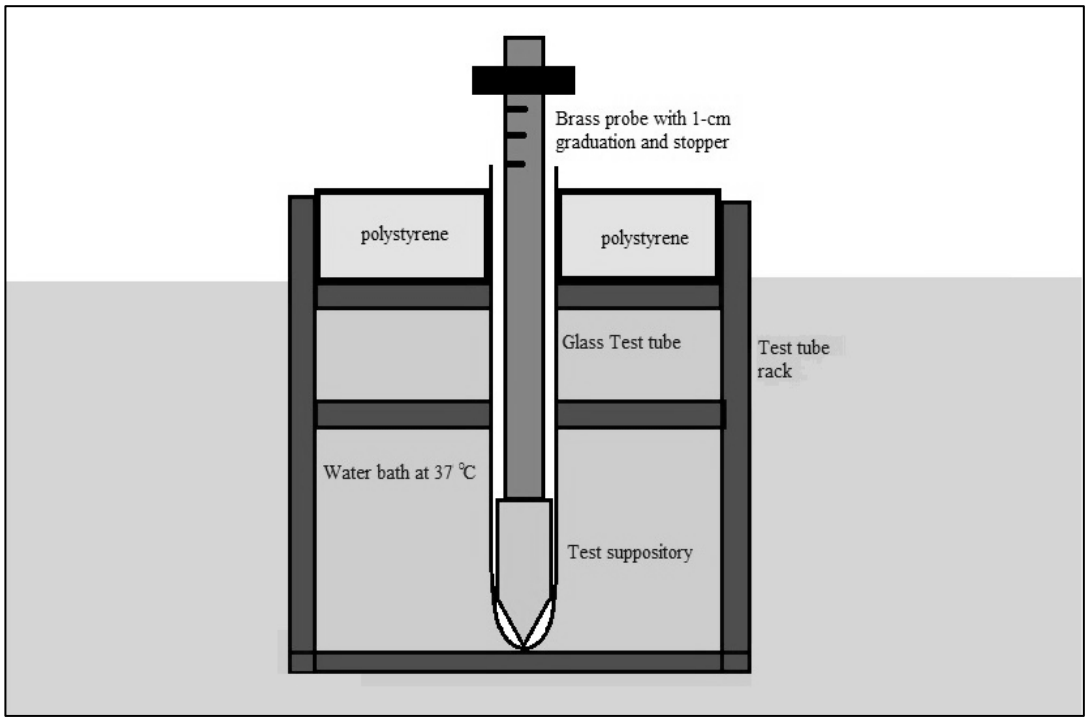


Figure 3.2 : The experimental setup of the softening point apparatus.

The setup of the apparatus illustrated in Figure 3.2, comprised of a rounded end pyrex test tube (inner diameter = 0.9 cm; length = 7.4 cm) (Iwaki, Japan) vertically immersed in a water bath maintained at 37.0 ± 0.5 ℃ using a test tube rack secured with a polystyrene holder. The polystyrene holder serves as an insulator to prevent heat loss as well as to secure the test tube at a 90° angle.

A suppository was introduced into the test tube pointed end first and a timer was started simultaneously as a graduated brass probe (diameter = 0.9 cm, length = 8.9 cm,

weight = 51.8 g) was placed over the suppository. The rod slowly penetrated the suppository as it softens. The end point (softening time) was measured as the time elapsed for the probe to penetrate 1 cm depth of the suppository. Experiment was carried out in triplicates for each formulation at room temperature of 24.5 ± 2.5 °C, and RH of 58 ± 5 %. Results were expressed as mean time ± SD.

3.3.2.8 Statistical analysis

The results obtained from Sections 3.3.2.5, 3.3.2.6 and 3.3.2.7 were analysed using analysis of variance (ANOVA) (SPSS Inc., version 20, USA). Post hoc analysis using Tukey's HSD test was carried out where appropriate when a statistically significant difference at $p < 0.05$ was obtained.

3.4 Results and discussion

3.4.1 Physical inspection

The visual appearances of the suppositories were acceptable for all the formulations. Suppositories were intact; surface was smooth; colour was even and uniformed with the absence of visible defects such as those illustrated in Figure 3.3 on the external surface of suppositories. There was no abnormal odour from the suppositories. Only suppositories which have passed the visual inspection were included in subsequent analysis.

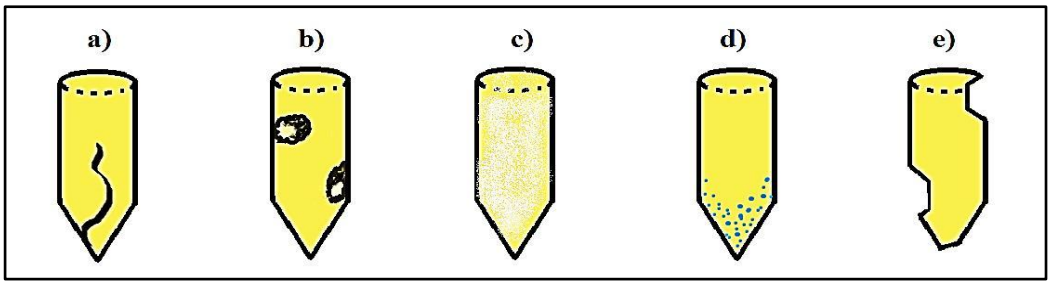


Figure 3.3 : The common defects found on suppositories: (a) fissures or cracks; (b) pitting; (c) fat-blooming; (d) particle sedimentation; (e) chipping.

3.4.2 Weight variation

The target weight, actual weight, melting point and content uniformity of the test formulations were tabulated in Tables 3.1-3.3 for CB, CE and SS respectively.

Suppositories produced weighed between 1.09 to 1.14 g (Tables 3.1-3.3). Mean actual weight of manufactured suppositories was in good agreement with the target weight, and deviations from respective target weights were ± 1 %. The SD value of the samples for each formulation was small (± 0.011 g), this was suggestive of good weight uniformity and that the additives were well dispersed within the suppositories. The British Pharmacopoeia (2015) stated that weight deviation in a batch of suppositories should be within the range of ± 5%. A large variation in weight could be due to either non-standardised mould cavity fill or non-homogeneity of molten mixture which would affect content uniformity of the suppositories (Mollel, 2006).

All batches of suppositories produced throughout this study were assessed for compliance to the weight uniformity (± 5 %) before further analysis. This provides assurance that suppositories used in subsequent analyses were homogenous and of satisfactory quality.

3.4.3 Melting point

Although thermal profiles of the investigated bases have been adequately characterised in Chapter 2, the melting point of suppositories produced may be altered by incorporation of additives (DcNa and bioadhesive polymers) or by the manufacturing process itself (El-Majri and Sharma, 2010; Mollel, 2006; Shegokar and Singh, 2010; Takatori et al., 2004; Yahagi et al., 1999). This study found that manufactured blanks for all three bases melted between 32.5 to 34.5 °C (Tables 3.1-3.3). Suppositories made using HPKS (CE and SS) melted at 1-2 ºC higher than the corresponding formulation produced using CB. The addition of DcNa did not appear to affect the melting point for suppositories made with all three bases (CB, CE and SS).

The addition of 1-5 %w/w CMCTS to CB suppositories increased melting point of the suppositories by 1 ºC, while addition of CMCTS decreased the melting point of CE suppositories slightly. SS suppositories containing PVP had consistently higher melting points compared to SS suppositories containing other bioadhesive polymers.

In general, the melting points of all suppositories containing 50 mg DcNa remained approximating body temperature; between 32.5 to 35.0 ºC. Yarnykh et al. (2011) reported that the melting point of commercial suppository bases found in the market ranged between 29.0 to 40.0 ºC. Suppositories produced using natural fatty bases, for example fat from borneo tallow seeds (Robertson, 1961) and palm oil blends (Pugunes and Ugandar, 2013) were found to have melting points ranging between 34.0 – 37.0 ºC and 37.0-37.1 ºC respectively.

Table 3.1 : The target weight, actual weight, melting point and DcNa content of CB suppository formulations containing 50 mg DcNa and bioadhesive polymers. Mean ± SD.

Polymers (%w/w)				**Target weight (g)**	**Actual weight (g) (n=10)**	**Melting point (°C)**	**DcNa content (mg) (n=3)**
CBP	HPMC	PVP	CMCTS				
CB without DcNa				-	1.090 ± 0.005	32.8	-
-	-	-	-	1.104	1.103 ± 0.005	32.7	48.51 ± 1.81
1	-	-	-	1.108	1.105 ± 0.005	32.9	-
2	-	-	-	1.112	1.109 ± 0.006	32.7	-
5	-	-	-	1.125	1.118 ± 0.006	32.5	48.52 ± 1.07
-	1	-	-	1.108	1.112 ± 0.004	33.1	-
-	2	-	-	1.111	1.111 ± 0.004	33.0	-
-	5	-	-	1.121	1.123 ± 0.004	33.0	49.52 ± 1.08
-	-	1	-	1.104	1.100 ± 0.006	32.8	-
-	-	2	-	1.104	1.101 ± 0.005	32.6	-
-	-	5	-	1.104	1.104 ± 0.004	32.8	49.25 ± 1.01
-	-	-	1	1.108	1.113 ± 0.006	33.7	-
-	-	-	2	1.112	1.117 ± 0.005	33.7	-
-	-	-	5	1.124	1.129 ± 0.006	33.8	49.28 ± 0.97

Table 3.2 : The target weight, actual weight, melting point and DcNa content of CE suppository formulations containing 50 mg DcNa and bioadhesive polymers. Mean ± SD.

Polymers (%w/w)				**Target weight (g)**	**Actual weight (g) (n=10)**	**Melting Point (°C)**	**DcNa content (mg) (n=3)**
CBP	HPMC	PVP	CMCTS				
CE without DcNa				-	1.101 ± 0.006	34.0	-
-	-	-	-	1.114	1.125 ± 0.006	34.4	47.97 ± 0.49
1	-	-	-	1.120	1.128 ± 0.009	34.3	-
2	-	-	-	1.122	1.126 ± 0.011	34.6	-
5	-	-	-	1.133	1.143 ± 0.006	34.2	48.17 ± 0.32
-	1	-	-	1.118	1.118 ± 0.005	34.7	-
-	2	-	-	1.121	1.121 ± 0.005	34.9	-
-	5	-	-	1.132	1.137 ± 0.009	34.7	49.07 ± 0.50
-	-	1	-	1.114	1.119 ± 0.009	34.8	-
-	-	2	-	1.114	1.120 ± 0.009	34.3	-
-	-	5	-	1.114	1.123 ± 0.011	34.4	49.39 ± 1.14
-	-	-	1	1.119	1.120 ± 0.006	33.6	-
-	-	-	2	1.121	1.128 ± 0.007	33.8	-
-	-	-	5	1.137	1.143 ± 0.005	33.9	49.03 ±0.24

Table 3.3 : The target weight, actual weight, melting point and DcNa content of SS suppository formulations containing 50 mg DcNa and bioadhesive polymers. Mean ± SD.

Polymers (%w/w)				**Target weight (g)**	**Actual weight (g) (n=10)**	**Melting point (°C)**	**DcNa content (mg) (n=3)**
CBP	HPMC	PVP	CMCTS				
SS without DcNa				-	1.098 ± 0.004	34.0	-
-	-	-	-	1.116	1.124 ± 0.006	34.3	48.55 ± 1.81
1	-	-	-	1.120	1.126 ± 0.005	34.8	-
2	-	-	-	1.124	1.131 ± 0.004	34.8	-
5	-	-	-	1.136	1.142 ± 0.007	34.6	47.53 ± 0.47
-	1	-	-	1.120	1.125 ± 0.008	34.4	-
-	2	-	-	1.123	1.129 ± 0.005	34.7	-
-	5	-	-	1.134	1.136 ± 0.010	34.8	48.33 ± 1.27
-	-	1	-	1.116	1.113 ± 0.007	34.9	-
-	-	2	-	1.116	1.120 ± 0.004	34.9	-
-	-	5	-	1.116	1.124 ± 0.004	35.1	48.84 ± 0.48
-	-	-	1	1.120	1.130 ± 0.005	34.7	-
-	-	-	2	1.124	1.129 ± 0.011	34.6	-
-	-	-	5	1.137	1.141 ± 0.005	34.5	48.68 ±0.85

3.4.4 Determination of DcNa content

This is especially important when suppositories are manufactured manually, as the manual process is highly variable. Quantification of DcNa content in selected samples obtained from a large batch allows verification of the labelled content as well as the uniformity of DcNa content between individual suppositories produced in a particular batch (Allen et al., 2008). Noordin and Chung (2004) investigated both the content uniformity between suppositories as well as within a suppository by comparing between different sections of a single dosage form to address uniformity of a suppository in the event of dose splitting. The active drug content of suppositories have to be within the range of 90-110% of the stated amount (British Pharmacopoeia, 2015). Content uniformity is achieved when not more than one suppository has drug content out of the 85-115 % range, and none outside the limit of 75-125% of the average content (British Pharmacopoeia, 2015).

The results of content uniformity studies for CB, CE and SS suppositories were summarized in Tables 3.1-3.3 respectively. All the formulations contained more than 95 % of the stipulated DcNa content and the SD values were ± 2 % of the average DcNa content. This confirmed the accuracy of DV determined in Section 2.3.3.3 which allowed proper adjustments of the amount of base required to account for different densities of additives added.

3.4.5 Viscosity

The bioadhesive polymers used in this study such as CBP and HPMC are known to have thickening properties, thus are likely to alter the viscosities of the bases determined in Section 2.3.1.5. Viscosity was generally found to be highest in CB

suppositories followed by CE and SS (Figures 3.4a-c). This could be due to the longer carbon chain of the palmitic (C_{16}) and stearic acid (C_{18}) in CB, compared to lauric acid (C_{12}) in CE and SS (Lonchampt and Hartel, 2004; Peyronel and Marangoni, 2014).

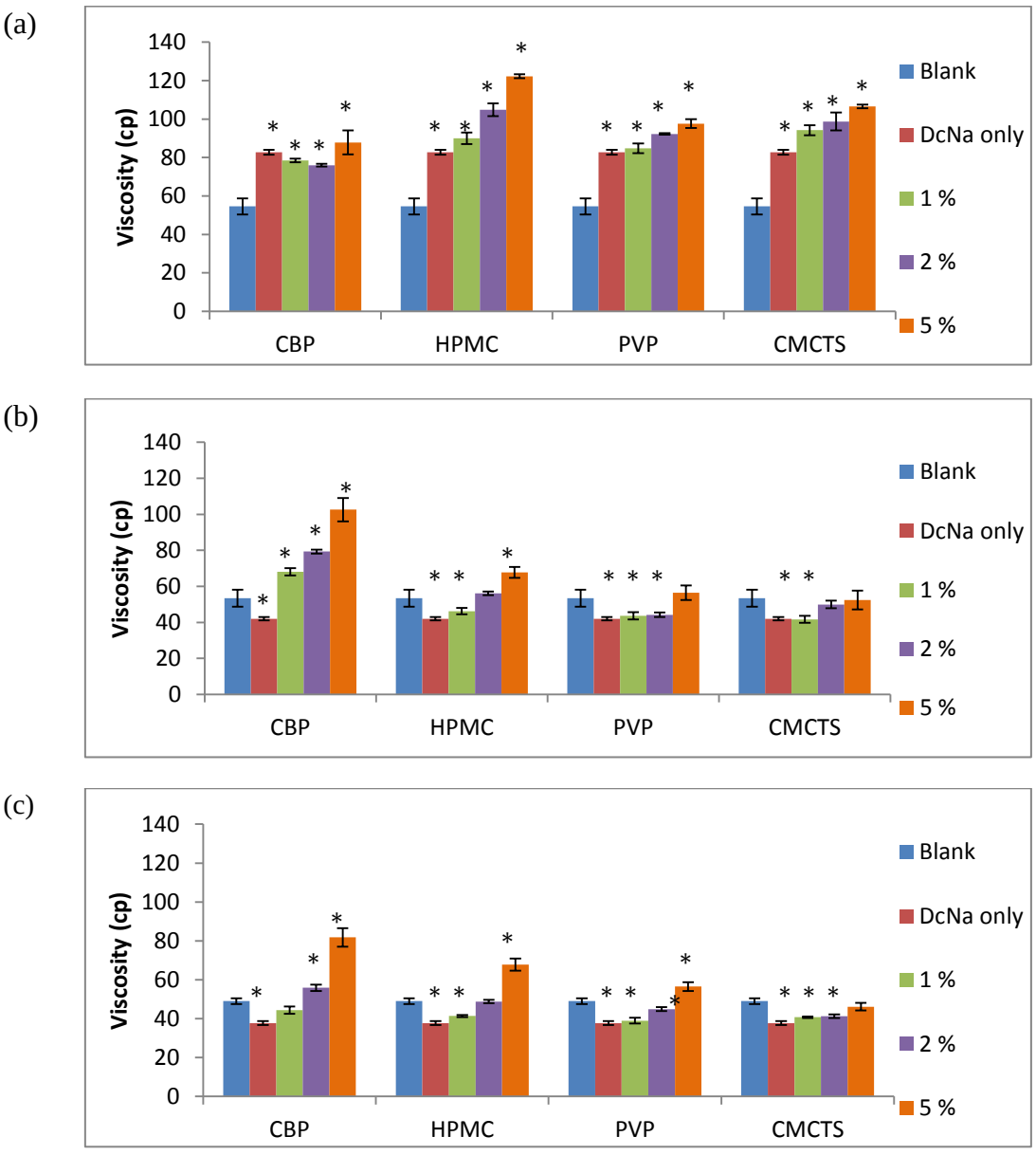


Figure 3.4 : The viscosity (cp) of suppositories made using (a) CB; (b) CE; and (c) SS; each suppository contained 50 mg DcNa and 1-5 %w/w of bioadhesive polymers (CBP, HPMC, PVP and CMCTS). Asterisks indicate formulations which are significantly different from blank suppositories.

The addition of DcNa significantly increased viscosity of CB suppositories but conversely, reduced the viscosity of CE and SS suppositories. Suppositories containing DcNa and bioadhesive polymer was generally more viscous than their corresponding DcNa only suppositories. CB suppositories incorporated with HPMC (Figures 3.4a) were most viscous while CE and SS suppositories incorporated with CBP (Figures 3.4b-c) were most viscous compared to suppositories with other bioadhesive polymers. The same polymer affected the viscosity of the suppositories differently. While PVP and CMCTS did not alter the viscosity of CE and SS suppositories drastically, it resulted in a significant concentration dependent increase in viscosity of suppositories when formulated with CB (Figures 3.4a-c). Generally, an increase in viscosity was observed when concentration of polymer is increased from 1 to 5 %w/w. Statistical comparison of the formulations using Tukey's HSD analysis is tabulated in Appendices 8-10.

More viscous suppositories may be beneficial to confine drug absorption within the lower rectum by limiting spread of the molten. However, excessively viscous suppositories may also impede DcNa release from the base and thus, drug release studies are required to determine if this increase in viscosity is unfavourable for drug release (Azechi et al., 2000).

3.4.6 Hardness

Hardness measurements obtained from the tip of suppositories were omitted from subsequent analysis as the results were less distinguishable between subsets (in Tukey's HSD analysis) compared to values obtained at the suppository barrel (Figures 3.5a-c). The values obtained depended on the crushed surface area at which the force

exerts on and this is made difficult by the cone-shaped pointed tip as the surface area varies along the length of the tip.

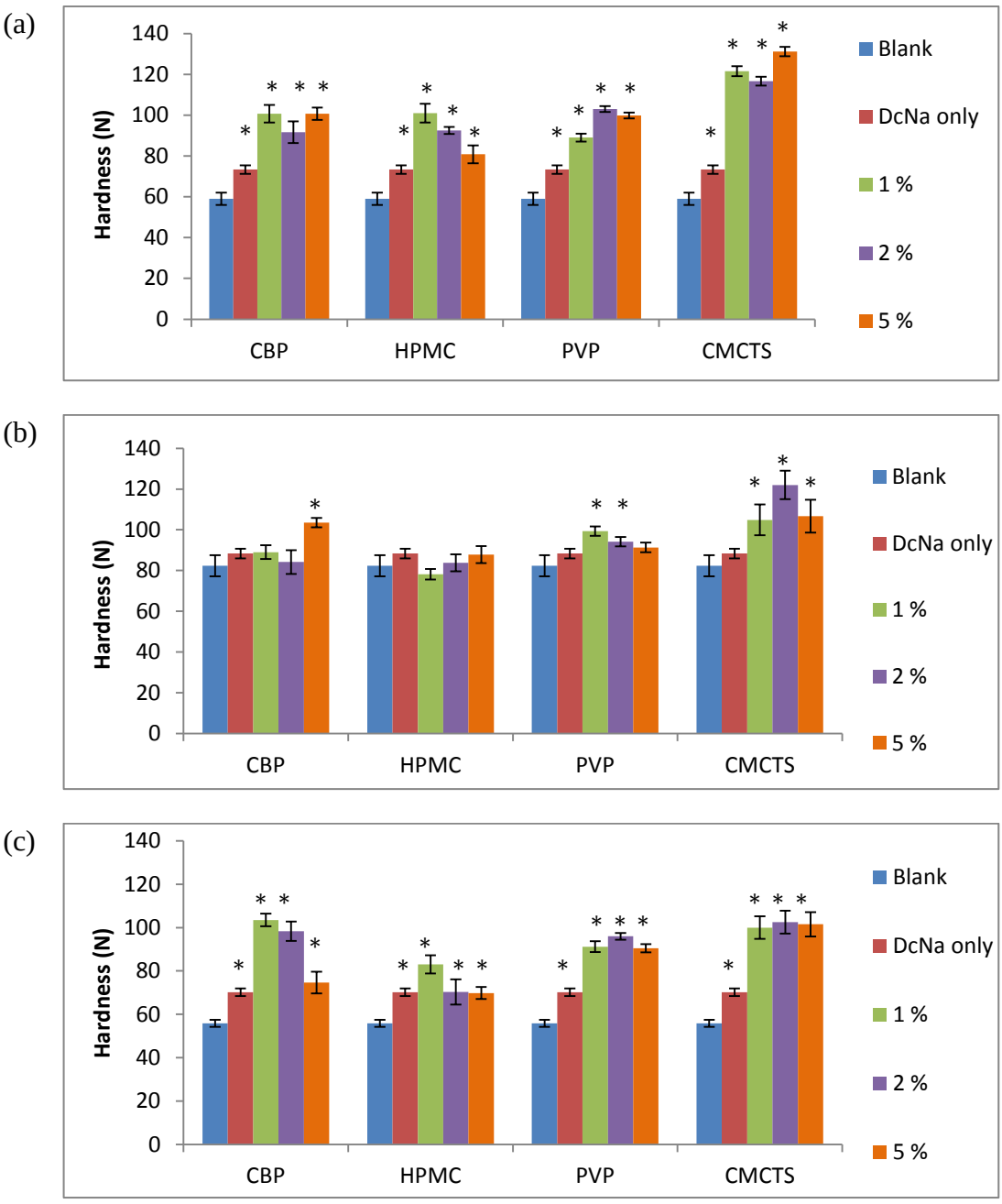


Figure 3.5: The hardness of suppositories produced using (a) CB; (b) CE and (c) SS; each suppository contained 50 mg DcNa and 1-5 %w/w of bioadhesive polymers (CBP, HPMC, PVP and CMCTS). Mean ± SD, n=6. Asterisks indicate formulations which are significantly different from blank suppositories.

Figures 3.5a-c showed that blank CE suppositories (82.33 ± 5.20 N) were significantly harder than the CB and SS suppositories (59.00 ± 2.97 N and 55.83 ± 1.60 N respectively). Addition of DcNa increased the hardness of all three (CB, CE, SS) suppositories. Statistical comparison of the formulations using Tukey's HSD analysis is tabulated in Appendices 11-13.

Suppositories incorporated with 1-5 %w/w of CMCTS for all three bases (CB, CE and SS) were hardest compared to those containing other polymers (Figures 3.5a-c). Addition of CBP showed an irregular hardness trend in all three bases. The presence of CBP significantly increased the hardness of CB suppositories; however the hardness of CE suppositories were not affected by addition of 1-2 %w/w CBP while SS suppositories were harder with 1-2 %w/w of CBP but not in the presence of 5 %w/w CBP (Figure 3.5c). On the other hand, formulations containing PVP were generally harder than those containing CBP and HPMC. HPMC suppositories were the least hard (Figures 3.5a-c). There was however, no observable trend between increment in polymer content and hardness of suppositories produced.

Sirisa-ard et al. (2014) found that suppositories produced using Krabok wax resulted in hardness of 6 N were too soft and easily broken, while Allen et al. (2008) suggested that suppositories should have hardness of 17-20 N. Meanwhile, studies by El-Majri and Sharma (2010); Oribe et al. (1995); Shegokar and Singh (2010) have reported a wide range of suppository hardness ranging from 10 to 60 N. These large differences of reported values were a result of differences in experimental methods as well as variability in the type of base and additives used. Although the addition of bioadhesive polymers have variable effects on the hardness of the bases, all the formulations in

this study had hardness values between 60 to 130 N, suggestive of good resistance towards the hazards of manufacturing and packing (Sirisa-ard et al., 2014).

3.4.7 Softening time

Figure 3.6 showed that all the formulations tested had a softening time between 3-7 minutes. Softening time of blank suppositories was found to be shortest in CB, followed by SS and CE; the post hoc Tukey's HSD test indicated that these outcomes were significantly different (Appendices 14-16). However, for suppositories containing 50 mg DcNa, the softening point of CB suppositories were increased while a decrease in softening time was observed for CE and SS suppositories.

In all the formulations containing polymers, softening time for CB suppositories were always faster than the corresponding formulations for CE and SS; both of which were comparable (CB < SS = CE). Generally, there was an increase in softening time with increasing polymer concentration. CE and SS suppositories incorporated with 5 %w/w HPMC showed the longest softening time among all the formulations tested.

The longer softening time in suppositories made using CE and SS (4-7 minutes) is an indication that the HPKS suppositories were slightly better at withstanding short exposures to heat compared to CB (3-5 minutes) suppositories. The British Pharmacopoeia (2015) has recommended that the softening time of lipophilic suppositories should be less than 30 minutes while studies by Janicki et al. (2001) and Moghimipour et al. (2009) produced suppositories with softening time which ranged between 4-13 minutes and 11-16 minutes respectively. Despite being harder than

formulations produced in similar studies (Section 4.3.6), all the formulations produced in this study had softening times below 7 minutes.

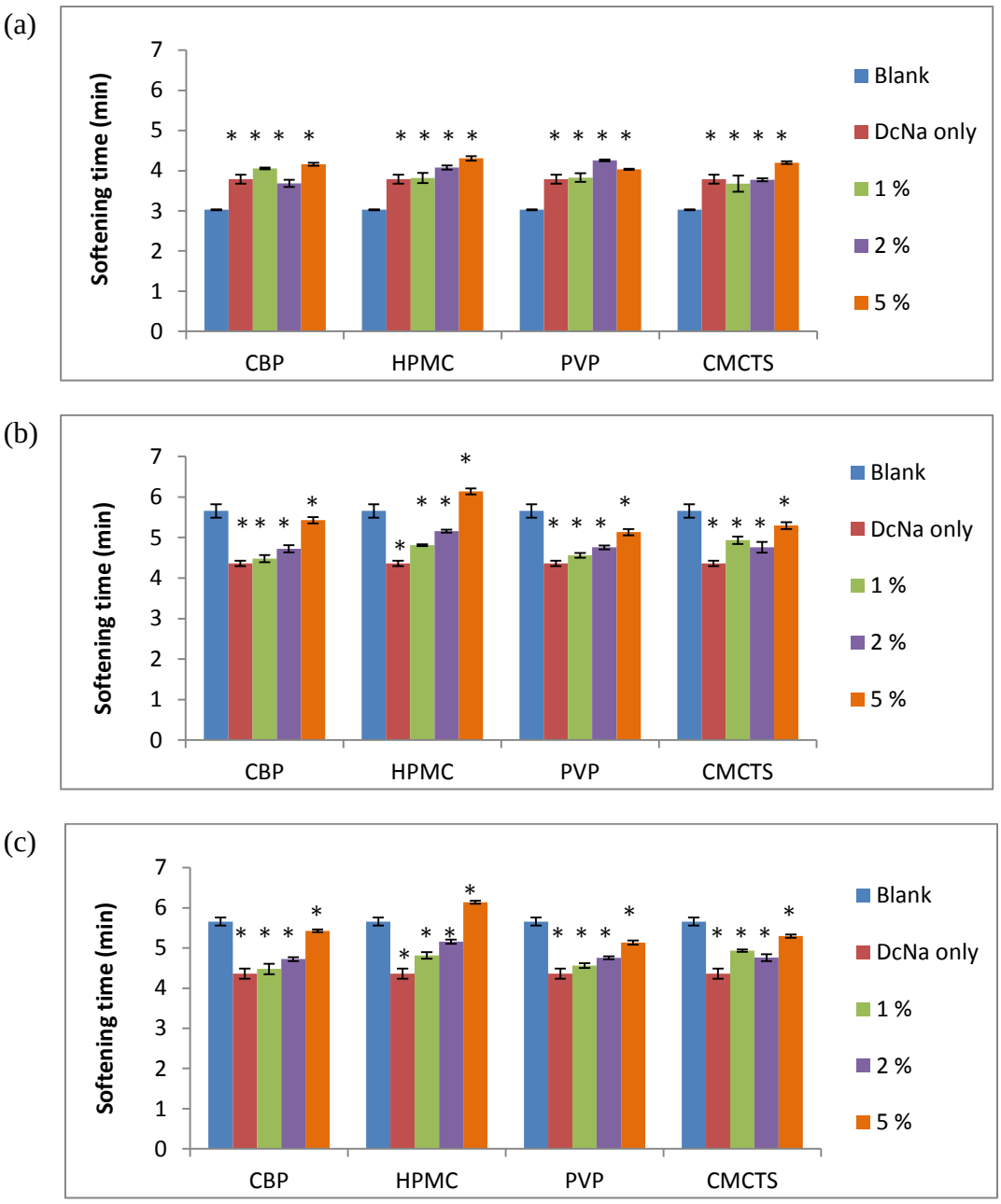


Figure 3.6 : The softening time of suppository formulations produced using (a) CB; (b) CE and (c) SS. Each suppository contained 50mg DcNa and 1-5 %w/w of bioadhesive polymers (CBP, HPMC, PVP and CMCTS). Mean ± SD, n=3. Asterisks indicate formulations which are significantly different from blank suppositories.

3.5 Conclusion

Suppositories containing bioadhesive polymers (CBP, PVP, HPMC, CMCTS) were successfully manufactured using CB and both the HPKS (CE and SS) as suppository bases through the optimised manufacturing methods from Section 2.3.3.

Dosage form analysis is essential for the evaluation of product quality. Suppositories manufactured in this study have good weight uniformity and there was minimal variation (± 1 %) between actual weight and the respective target weight. The melting point of suppositories manufactured using CE and SS were 1-2 ºC higher than CB suppositories but all formulations had melting points within the range of 32.5 °C to 35.5 °C. The addition of bioadhesive polymers did not significantly alter melting point (less than ± 1 °C) of suppositories, maintaining a melting point close to body temperature which is suitable for rectal administration. All the formulations tested contained > 95 % of the stipulated DcNa content.

In general, there was no consistent trend between hardness and increasing concentrations of bioadhesive polymers in the suppositories (CB, CE and SS) based on the post hoc Tukey's HSD analysis ($p < 0.05$). Conversely, viscosity of the molten suppositories increased with increasing amount of bioadhesive polymers (CBP, PVP, HPMC, CMCTS) incorporated into the formulations. Softening times on the other hand, were longer in suppositories produced using the HPKS (CE and SS) than CB. However, the softening times for all formulations were between 3–7 minutes which was acceptable for rectal drug administration.

Physical properties of the suppositories examined in this chapter were found to be suitable for rectal administration. The longer softening time in HPKS suppositories coupled with a melting point of 34.5 ± 1 °C is an added advantage compared to CB suppositories. This offers more resistance to melting or damage during handling prior to insertion into the rectum. Although the physical properties are well characterised in this chapter, further studies are required to examine the impact of various additives on the drug release (Chapter 4) and bioadhesive properties (Chapter 5).

CHAPTER 4

IN VITRO DRUG RELEASE AND RELEASE KINETICS

4.1 Introduction

4.1.1 Drug release studies

After suppository administration into the rectum, the base has to first melt to allow diffusion of drug from the base into rectal environment before absorption through rectal mucosa into systemic circulation (Allen et al., 2008). Previous studies have found that data obtained from *in vitro* drug release studies for suppositories were well correlated to plasma concentration profiles (Aiache et al., 1987; Babar et al., 1999; Chicco et al., 1999; Gjellan et al., 1994; Vidras et al., 1982). Therefore, these studies are useful for prediction of dosage form performance.

Various methods exist for studying *in vitro* drug release from suppositories. Among those commonly used are the USP paddle apparatus (Ahmad, 2001; Moghimipour et al., 2009; Young et al., 1987), basket apparatus (Babar et al., 1999; Ermiş and Tarimci, 1995; Gjellan et al., 1994; Swamy et al., 2012), dialysis rotating cell method (Aoyagi et al., 1988; Lootvoet et al., 1992; Oribe et al., 1995), Muranishi method (Ermiş and Tarimci, 1995; Umeda et al., 1985), and flow through cell method (Aiache et al., 1987; Mollel, 2006; Tukker et al., 1981; Yahagi et al., 1999).

Apart from type of apparatus; the type, pH and volume of dissolution medium used; temperature of the *in vitro* run as well as method of quantification of active drug has to be carefully selected based on the type of dosage form studied.

4.1.2 Statistical comparison and mathematical modelling

The three main methods used to analyse data obtained from drug release studies are: (1) exploratory data analysis; (2) model–independent and; (3) model-dependent methods.

4.1.2.1 Exploratory data analysis

As the term suggests, exploratory data analysis is used during initial interpretation of raw data to provide basic understanding of how drug is released from a formulation. This method compares drug release both graphically and numerically.

In graphical comparison, cumulative drug release against time plots were visually inspected for overlapping of error bars (extending two standard errors (SE) either side of the mean) between the drug release profiles at each time point. Non overlapping of error bars indicates that drug release profiles at that particular time point are significantly different from each other (O'Hara et al., 1998; Yuksel et al., 2000).

Exploratory data analysis can also be conducted numerically by summarising data in the form of 95 % confidence interval for the difference in the mean drug release profiles at each time point. Difference at a particular time point is considered significant when the $p > 0.05$ (O'Hara et al., 1998).

4.1.2.2 Model-independent methods

This method involved computing of raw data into mathematical formulae to obtain single-value measurements of differences between two multiple point drug release

profiles. This is further differentiated into amodelistic dissolution parameters (ratio test) and pair-wise procedures (Costa and Sousa Lobo, 2001; O'Hara et al., 1998).

Amodelistic parameters compare profiles using dissolution efficiency (DE) and mean dissolution time (MDT) (Costa and Sousa Lobo, 2001; Mollel, 2006; Vaghela et al., 2011), both determined using the following equations:

Equation 4.1

$$DE = \frac{\int_{t_1}^{t_2} y \; dt}{y_{100} * (t_{2-} t_1)} \; x\,100\%$$

Where,

y = percentage of drug released at time t_2

y_{100} = maximum amount of drug available for release

Equation 4.2

$$MDT = \sum_{i=1}^{n} \hat{t}_i \frac{M_t}{M_\infty}$$

Where,

n = number of dissolution samples

i = sample number

$\hat{t}_i$ = time at midpoint between t_i and t_{i-1}

M_t = fraction of dose released at time t_i

M_∞ = dose of formulation

Pair-wise procedures compare profiles in terms of 'fit factors' and Rescigno's indices (ξ). The 'fit factors' or 'similarity indices' known as 'difference factor' (f_1) and the

'similarity factor' (f_2), both described by Moore and Flanner using Equation 4.3 and Equation 4.4 (Anderson et al., 1998; Costa, 2001; Moore and Flanner, 1996).

Equation 4.3
$$f_1 = \left\{ \frac{\sum_{t=1}^{n} w_t \left| R_t - T_t \right|}{\sum_{t=1}^{n} w_t R_t} \right\} x\ 100$$

Equation 4.4
$$f_2 = 50\, log_{10} \left\{ \left[1 + \frac{1}{n} \sum w_t \left(R_t - T_t \right)^2 \right]^{-0.5} x\ 100 \right\}$$

Where,

R_t	=	percentage of drug release for reference sample
T_t	=	percentage of drug release for test sample
n	=	number of sampling points taken into account
w_t	=	optional weight factor which is usually kept at 1

The f_1 represents the average percentage difference between test formulation (T_t) and reference formulation (R_t) across all time points analysed while the f_2 is a log function of differences between the compared profiles and can assume any value between 0 and 100. The Food and Drug Administration (FDA) has set a standard where f_1 between 0 to 15 and f_2 between 50 to 100 indicate similarity between two drug release profiles (Anderson et al., 1998; Costa and Sousa Lobo, 2001; Ministry of Health Malaysia, 2000).

4.1.2.3 Model–dependent methods

In the model-dependent approach, data obtained from drug release studies are fitted into selected mathematical equations describing release kinetics. Various parameters are generated to determine goodness of fit of data to the selected kinetic equation. These parameters include release constant, linear coefficient of determination (r^2) and lag time which describes the rate of release as well as the proximity of data to the release model tested. It was suggested that this approach requires a minimum of four data points excluding time point 'zero' up till 80 % of drug release or when asymptote is achieved (O'Hara et al., 1998; Yuksel et al., 2000).

4.1.2.3.1 First order kinetics

First order release kinetics describes drug release in a concentration-dependent manner, this was first described by Gibaldi and Feldman (1967) based on the Noyes-Whitney equation where drug release occurs at a constant proportion to the amount remaining within the dosage form (Costa and Sousa Lobo, 2001). This kinetic model has been used to describe release of water soluble drugs from porous matrices, and also lipophilic suppositories (Tarimci and Ermis, 1997). First order release is explained by the following equation (Basak et al., 2008; Dash et al., 2010);

Equation 4.5

$$log_{10}\, C_t = log_{10}\, C_0 - \frac{K_1 . t}{2.303}$$

Where,

Ct = amount of drug remaining to be released at time t

C_0 = initial amount of drug in the dosage form (usually 100 %)

K_1 = first order rate constant

4.1.2.3.2 Higuchi release

The Higuchi model was initially developed to quantify mass transport of homogenously dispersed drug particles in a planar matrix into perfect sink conditions based on Fick's Law (Dokoumetzidis and Macheras, 2006; Kalam et al., 2007; Siepmann and Peppas, 2011). This model can be described by the simplified equation (Costa and Sousa Lobo, 2001);

Equation 4.6 $$Q_t = K_H . t^{1/2}$$

Where,

Q_t = amount of cumulative drug released at time t

K_H = Higuchi release constant

This equation assumes that drug release occurs in a thin, planar geometry, where drug movement through the matrix (base) is a rate limiting step, provided that (1) amount of drug in the dosage forms greatly exceeds drug solubility to achieve pseudo-steady-state and, (2) the base does not swell or dissolve during drug release so that distance of the base-medium interface remains unaltered (Siepmann and Peppas, 2011). The Higuchi equation is only valid for analysis of the first 60 % of drug release.

Previous studies on drug release from lipophilic suppositories have suggested that the release were in agreement with Higuchi equation (Nakajima et al., 1990; Takatori et al., 2004). This equation could be useful to describe drug release from suppositories as the suppositories melt and form a thin layer of molten base matrix containing suspended drug particles, similar to that observed in the ointment-skin interface from which the Higuchi equation stems from.

4.1.2.3.3 Korsmeyer – Peppas model

This mathematical model was initially developed by (Korsmeyer et al., 1983) to explain diffusion-controlled drug release mechanisms from polymeric systems, and subsequent studies lead to characterisation of the type of diffusional release based on the release exponent 'n', taking into account variable geometries and swelling capacities of dosage forms. A number of lipophilic suppositories demonstrated release patterns with good fit to the Korsmeyers-Peppas model (Güneri et al., 2004; Mollel, 2006; Uzunkaya and Bergişadi, 2003). The release kinetics can be described as,

Equation 4.7

$$\log \frac{M_t}{M_\infty} = \mathrm{n}.\log t \; + \; \log k_m$$

Where,

M_t / M_∞ = fraction of drug released at time t

K_m = release constant

n = release exponent

In suppositories (non-swellable cylindrical dosage forms); the 'n' value limits of n = 0.45 for Fickian diffusion; $0.45 < n < 1.0$ non- Fickian or anomalous diffusion; $n \geq 1$ for case-2 transport (zero order release) were adopted.

4.1.2.3.4 Weibull model

The Weibull model has been used empirically to describe drug release from Euclidian and fractal matrices; despite criticism of the lack of kinetic basis and non-dissolution specific nature of its parameters (Costa et al., 2003). Use of Weibull function in drug release stemmed from the concept where a concentration gradient is present at the

releasing boundaries of dosage forms, which are either described as a Euclidian matrix or fractal geometry (Kosmidis et al., 2003). The Weibull model is described by the following equation;

Equation 4.8

$$m = 1 - \exp(\frac{-(t - T_{lag})^{\beta}}{\alpha}$$

Where,

m	=	amount of drug released
α	=	scale parameter (rate constant)
β	=	shape parameter
T_{lag}	=	lag time

Description of drug release using the Weibull model is through the scale parameter (α) and shape parameter (β) which represents the apparent rate of release and shape of the curve respectively. When β=1, the curve corresponds exactly to a homogeneous model similar to that of first order kinetics; while β>1 indicates a sigmoidal curve with an inflexion point; and β<1 indicates a steeper initial slope than exponential curves (Cupera, 2009; Dash et al., 2010).

4.1.2.3.5 Bi-exponential first-order kinetic model

This model had been used to describe drug release from fenbufen suppositories made using lipophilic, hydrophilic and ampiphilic bases as well as in sustained release indomethacin tablets and capsules (Laakso et al., 1984; Young et al., 1987). The equation defining bi-exponential first-order kinetics is as the following;

Equation 4.9 $$w = Ae^{-k_1 t} + Be^{-k_2 t}$$

Where,

w	=	drug remaining to be released at time t
k_1	=	release rate constant for initial phase
k_2	=	release rate constant for terminal phase
A	=	amount of drug released in initial phase
B	=	amount of drug released in terminal phase

The plot of remaining drug against time would generate a biphasic curve. Parameters generated from linear regression would yield release constants, k_1 and k_2 which reflects rate of release at each phase and their respective coefficient of determination (r^2) which indicates closeness of fit to the equation while the y-axis intercept corresponds to lag time. A good fit to the equation is represented by r^2 approximating to 1 and small, reasonable lag times (Laakso et al., 1984).

4.1.3 Selection of the best fit model

To determine the best fit model, previous studies employed coefficient of determination (r^2) or adjusted coefficient of determination (r^2 adjusted) (Ladani et al., 2011), sum of squares residues (SSR) (Thakkar et al., 2009), mean square error (MSE), Akaike Information Criterion (AIC) (Ozkan et al., 2000) and more recently the ratio of SSR/r^2 (Costa and Sousa Lobo, 2001; Singh et al., 2011). The r^2 measures proportion of variation between observed data and the mean generated through linear regression model. The greater approximation of r^2 to 1, the closer the fit of data to a particular model (Ladani et al., 2011). The AIC, on the other hand, shows the quality

of fit by comparing models using the same weighting scheme. The model which produces the smallest AIC value is regarded to provide the best fit out of a set of models tested (Costa, 2001).

Within the context of this research project, *in vitro* drug release studies of DcNa from the HPKS bases (CE and SS) were essential as the suppository bases used were non-conventional. There were no reported data on drug release characteristics from these bases and how they fared compared to CB. Furthermore, the inclusion of a bioadhesive component in the form of polymers could alter drug release profiles. Thus, this chapter aims to quantify DcNa release from the formulation prototypes developed in Chapter 3 using suitable dissolution study methods, followed by comparison of DcNa release kinetics from the formulations using exploratory data analysis methods, model independent methods and mathematical models. This chapter will also assess the effects of bioadhesive polymers (CBP, HPMC, PVP and CMCTS) on drug release kinetics.

4.2 Materials and methods

4.2.1 Dissolution setup and evaluation of formulations

4.2.1.1 Experimental setup

The *in vitro* release of DcNa from suppositories was studied using the 8-vessel Distek dissolution system 2100c (Distek Inc., New Jersey, USA) fitted with thermocirculator TCS 0200 (Distek Inc., New Jersey, USA). The system is connected to Distek Evolution 4300 syringe pump dissolution sampler (Distek Inc., New Jersey, USA).

Suppositories were enclosed in helix-shaped sinkers made using steel wires with dimensions (length 2.8 cm x diameter 1.0 cm; interloop distance of 0.5 cm) to ensure retention of suppositories at the bottom of the vessel. Dissolution of a blank suppository made with only the base (CB, CE and SS) was used as experimental blank. The parameters employed during dissolution studies were summarized in Table 4.1. Samples were then analysed using a single cell UV-Visible spectrophotometer (Libra, S12) at a wavelength of 276 nm as determined in Chapter 2.

Table 4.1 : Summary of parameters used for *in vitro* drug release studies.

Experimental Parameters	**Settings**
Dissolution tester	USP Apparatus II – Paddle
Dissolution media	Distilled water
Volume of media	900 mL
Temperature	37.0 ± 0.5 °C
Paddle rotation speed	50.0 ± 0.2 rpm
Filter pore size	0.45 μm
Replicates	6 suppositories per experiment
Sampling time (min)	2, 6, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360
Sampling volume (mL)	5 mL, reconstituted

4.2.1.2 Evaluation of formulations

4.2.1.2.1 The effects of drug loading on DcNa release

The effects of drug loading on DcNa release was evaluated using suppositories made from all three bases (CB, CE, SS) containing 25, 50 and 75 mg DcNa using procedures mentioned in Section 4.2.1.1. Data obtained was analysed using exploratory data analysis method.

4.2.1.2.2 The effects of bioadhesive polymers on DcNa release

DcNa release from the formulations containing bioadhesive polymers (CBP, HPMC, PVP, CMCTS) at concentrations of 1-5 %w/w were studied using methods described in Section 4.2.1.1. A suppository containing the corresponding amount of bioadhesive polymers without DcNa was used as experimental blank. Comparisons were carried out via model-independent methods and model dependent methods.

4.2.2 Statistical and mathematical analysis of data

4.2.2.1 Exploratory data analysis

Graphical comparison was employed to analyse DcNa release from suppositories (CB, CE and SS) incorporated with 25, 50 and 75 mg of DcNa. Percentage cumulative drug release was plotted against time with error bars indicative of two SE at both sides of the error bar (95 % confidence interval) at each time point (Mollel, 2006; O'Hara et al., 1998). The graph is then visually inspected for overlapping of error bars.

4.2.2.2 Model-independent method

The fit-factors (f_1 and f_2) were used to identify dissimilar drug release profiles by comparing the T_t formulations containing bioadhesive polymers (CBP, HPMC, PVP, CMCTS) and their corresponding R_t (suppositories containing only base and DcNa). Meanwhile, other model-independent parameters such as DE and MDT were used to describe the rate and extent of DcNa release from both R_t and T_t suppositories.

4.2.2.3 Model dependent method

Drug release data from all formulations were fitted into first order, Higuchi's release, Korsmeyer–Peppas model, Weibull's equation and biphasic first-order release

equations using KinetDS® software version 3.0 (Aleksander Mendyk, Kraków, Poland.). Goodness of fit for the various models were investigated and compared in terms of r^2 and AIC values.

4.3 Results and discussion

4.3.1 Method development

While the United States Pharmacopeia and National Formulary, (2008) and European Pharmacopoeia, (2010) specified the use of paddle dissolution apparatus and flow through cell apparatus respectively to study drug release from suppositories; various alternatives have been attempted (Gjellan and Graffner, 1994; Nair and Bhargava, 1999; Palmieri, 1981; Webster et al., 1998). Palmieri (1981) however, reported erratic and irreproducible results using the basket apparatus due to clogging of basket mesh by melted base; while Gjellan and Graffner (1994) found that the flow through cell resulted in more rapid drug release with a larger variance in data compared to basket and paddle apparatus.

The rectal environment is simulated by the receptor medium during *in vitro* drug release studies. Although it should mimic physiological environment closely, it is impractical to carry out drug release studies under stringent rectal physiological parameters especially when only 2-3 mL of mucus is present in the rectum. The use of 2-3 mL receptor media will not be able to provide 'sink' conditions for drug release which should be at least 3 times the solubility of the drug tested (Brown et al., 2011; Lee et al., 2008; Vaghela et al., 2011).

Meanwhile the rectal pH also affects ionisation of drug and its partitioning out of the base which could alter drug release. pH of the rectum varies with age and has been reported to be within the range of 6.29–6.45 in neonates; 6.68–7.12 in infants older than 28 days while rectal pH in children aged between 1 to 14 years ranged between 7.2-12.1 (Jantzen et al., 1989; Turner et al., 2012). Adult rectal pH on the other hand is approximately 7.2, but varies according to colonic content (Desai, 2007). Since the rectum is void of buffering capacity; use of a buffered medium would not reflect actual drug release conditions within the rectum, especially when aqueous solubility of DcNa is pH dependant (Chuasuwan et al., 2009).

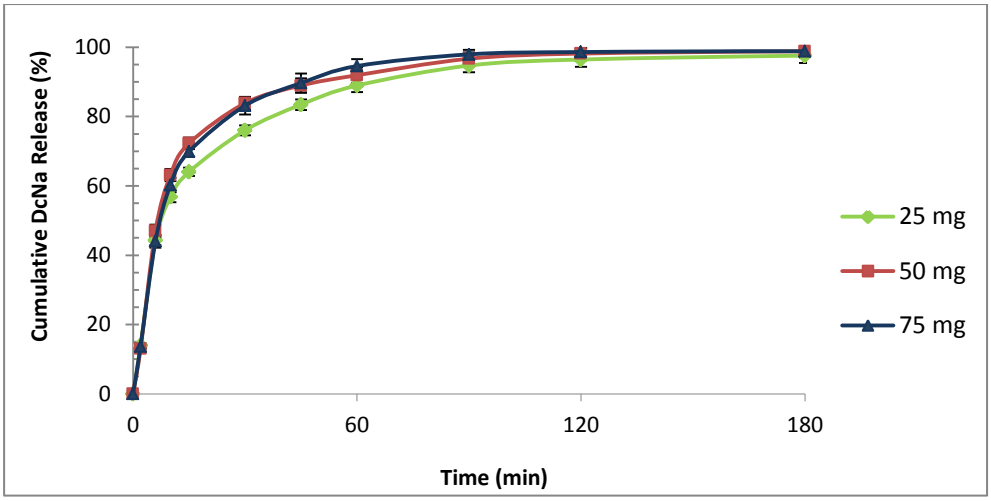
After careful considerations of the factors mentioned above, this research employed the USP II paddle dissolution apparatus with the use of sinkers due to considerations that lipophilic bases and bioadhesive polymers used are likely to clog the basket apparatus mesh. Distilled water maintained at 37.0 ± 0.5 °C was selected as the receptor medium for drug release studies to reflect lack of buffering capacity and temperature within the rectum (Allen et al., 2008; Bottger et al., 1989; Grayson, 1951).

4.3.2 Exploratory data analysis

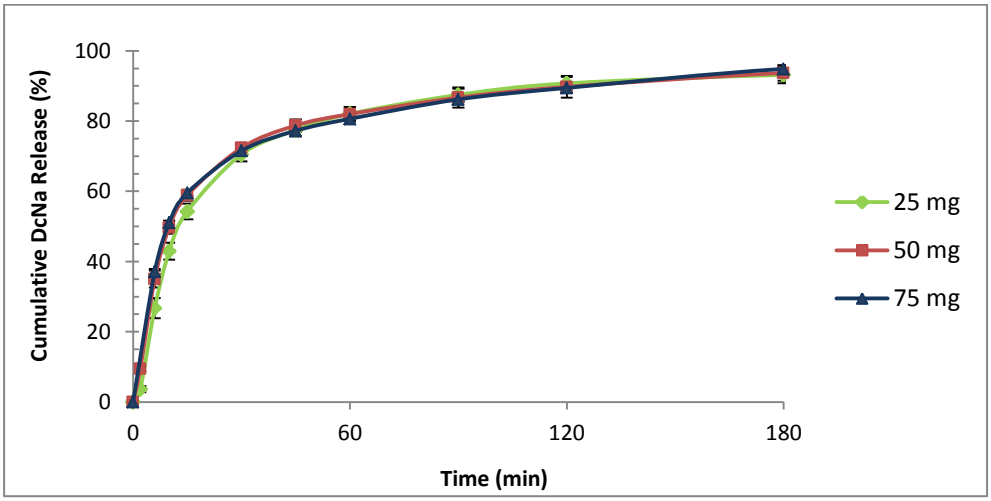
4.3.2.1 The effects of drug loading on DcNa release

Drug release profiles of suppositories incorporated with 25, 50 and 75 mg of DcNa were superimposable upon visual inspection for all the three bases (CB, CE and SS) investigated (Figures 4.1a-c). However, the exploratory analysis method produced inconclusive results as there was overlapping of error bars at certain time points (no significant difference) while others were significantly different.

(a)



(b)



(c)

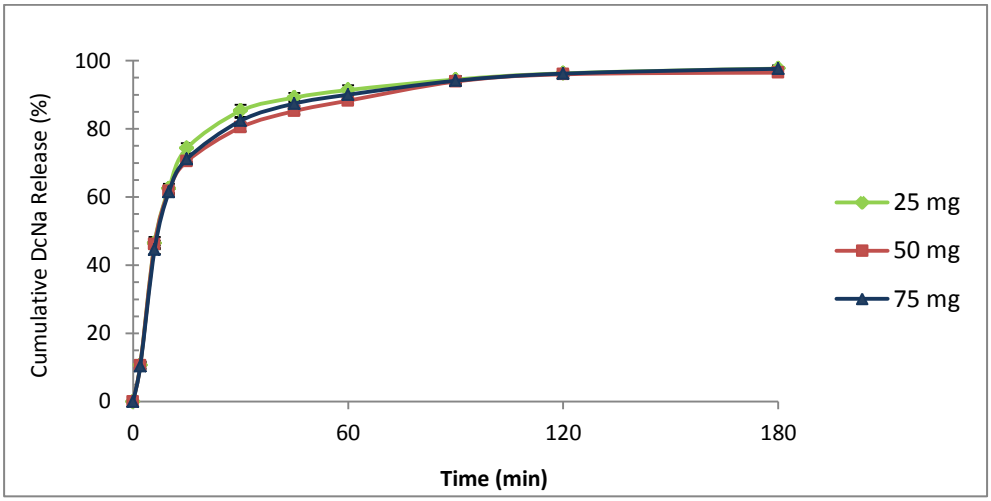


Figure 4.1 : Cumulative percentage release of DcNa in (a) CB; (b) CE; (c) SS suppositories containing 25, 50 and 75 mg of DcNa. Mean ± 2 SE, n=6.

Further ANOVA analysis followed by post hoc Tukey's test showed that extent of DcNa release from bases was not affected by initial amount of DcNa incorporated. Figure 4.1a showed that although CB suppositories with 25 mg DcNa had significantly lower initial rate of release from 10 to 60 minutes compared to those with 50 and 75 mg DcNa ($p< 0.05$), the extent of DcNa release was indifferent at 180 minutes ($p = 0.155$).

CE and SS suppositories containing 25 mg DcNa on the other hand, had significantly lower release between 6 to 15 minutes ($p< 0.05$) and 15 to 60 minutes ($p< 0.05$) respectively compared to the corresponding formulations containing 50 and 75 mg of DcNa (Figures 4.1b-c). The extent of DcNa released at 180 minutes was not significantly different between the three CE formulations ($p= 0.977$) and the three SS formulations ($p= 0.06$). This indicated that while drug load did not affect the extent of DcNa release; the initial rate of release was increased when drug loading is doubled from 25 mg to 50 mg, but this effect was not significant when drug loading was further increased to 75 mg.

This was further supported by the plot of actual DcNa released (Appendix 17) as each time point interval showed that there was rapid release of DcNa during the first 45 minutes for all the suppositories. The actual amount released at each time interval increased with increasing amount of DcNa incorporated ($p< 0.05$). This suggested that initial release of DcNa did not occur at a fixed rate (zero order). The anomalous increase in actual amount of DcNa released at time point interval of 30 minutes could be a result of change in surface area for DcNa release due to melting and molten accumulation at the dissolution media-air interface in the dissolution vessel. Further

investigation on kinetics of DcNa release was conducted using mathematical models (Section 4.3.4).

Comparing DcNa release from suppositories containing 50 mg DcNa (Figure 4.2), release profiles between CB and SS were not significantly different up to 240 minutes while the profiles for CE were significantly different from CB and SS up to 120 minutes ($p < 0.05$). The release plateaued after 120 minutes in both CB and SS while it was only achieved after 180 minutes in CE suppositories.

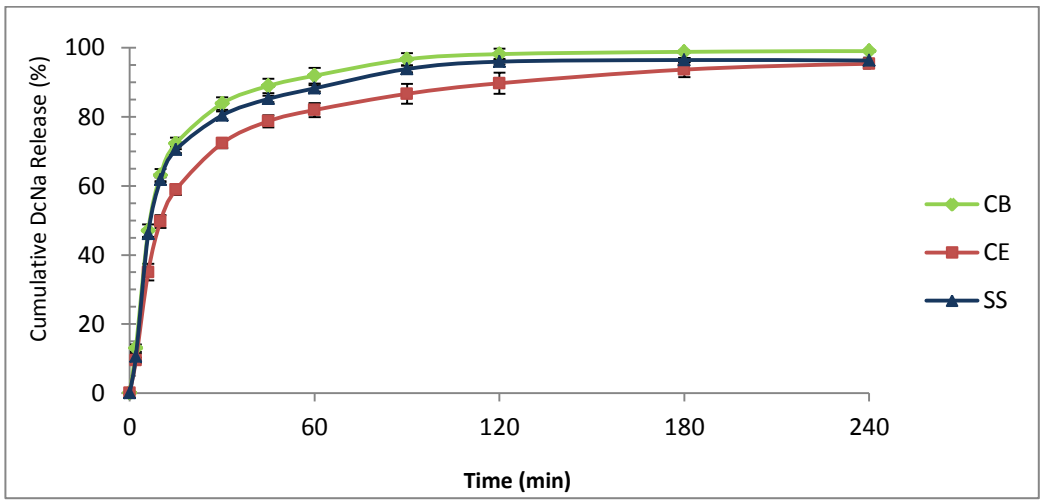


Figure 4.2 : DcNa release profiles from suppositories made with different bases, each containing 50 mg of DcNa. Mean ± 2 SE, n=6.

CB, CE and SS suppositories containing 50 mg DcNa released more than 50 % DcNa within the first 10 minutes, and more than 80 % within the first 60 minutes of dissolution. Although there were no pharmacopoeia requirements for qualification as fast-acting suppositories, the United States Pharmacopeia and National Formulary (2008) required fast-acting tablets to achieve at least 75 % of drug release within the

first 60 minutes of dissolution. CB, CE and SS suppositories were found to provide fast release of DcNa upon dissolution.

The ANOVA analysis with post hoc Tukey's test used in this section was used to provide descriptions for the observations via exploratory method of data interpretation. However, this method was found to be tedious and generated large quantities of data based on point-to-point comparisons between two profiles. The method did not consider drug release process as a whole but provided only comparison at a specific time point which limits its practicality when more than two time points or release profiles are being compared.

4.3.3 Model- independent method

Due to difficulties in simultaneous interpretation of multiple drug release profiles using methods in Section 4.3.2, fit factors (f_1 and f_2) were used to compare dissimilarities between dissolution profiles in the course of this study. Subsequently, DE and MDT were calculated for evaluation of the extent and rate of DcNa release of each formulation. The corresponding drug release profiles were attached in Appendices 18-21 for reference.

4.3.3.1 Effects of bioadhesive polymers on DcNa release

4.3.3.1.1 Fit factors (f_1 and f_2)

This method had been endorsed by both FDA and Malaysian Ministry of Health for model-independent comparison of drug release profiles (Ministry of Health, 2000; US Department of Health and Human Services & CDER, 1997).

Comparisons using fit factors (Table 4.2) were made between the R_t which contains only 50 mg DcNa, against T_t which are incorporated with 1-5 %w/w bioadhesive polymer. Table 4.2 showed that drug release profiles between CB-SS suppositories and CE-SS suppositories appear more similar than CB-CE. The addition of CBP increasingly altered drug release profiles with increasing polymer concentrations, resulting in $f_1 > 15$ and $f_2 < 50$ for suppositories made with all three bases.

Generally, incorporation of 1-5 %w/w HPMC, PVP and CMCTS to CE and SS did not alter drug release profiles compared to their respective R_t. In CB suppositories, the addition of 1-2 %w/w HPMC, PVP and CMCTS did not alter dissolution profiles; but when concentration of polymer was increased to 5 %w/w, DcNa release for all three formulations were significantly different from R_t ($f_1 > 15$ and $f_2 < 50$).

Although fit factors were effective in describing the similarities and dissimilarities between profiles, it was unable to reflect the reason of dissimilarities in terms of rate and extent (maximum amount) of drug release or kinetics of drug release. In order to understand DcNa release from these suppositories, it is thus essential to assess the DE (Section 4.3.3.1.2) and MDT (Section 4.3.3.1.3) for evaluation of the extent and rate of DcNa release from each formulation.

Table 4.2 : Statistical comparison of formulations containing DcNa only (R_t) and formulations with DcNa and bioadhesive polymers (T_t). Dissolution profiles are considered similar when; $0 < f_1 < 15$ and $50 < f_2 < 100$. The highlighted f_1 and f_2 values indicate similar dissolution profiles between R_t and T_t.

Formulation						**Parameters**	
R_t	**T_t**						
Base		**Polymer amount (%w/w)**					
		CBP	**HPMC**	**PVP**	**CMCTS**	f_1	f_2
CB	CE	0	0	0	0	17.389	47.450
CB	SS	0	0	0	0	3.729	78.410
CE	SS	0	0	0	0	14.652	52.490
CB	CB	1	-	-	-	36.418	29.433
CB	CB	2	-	-	-	49.455	23.911
CB	CB	5	-	-	-	88.266	1.867
CB	CB	-	1	-	-	9.861	55.968
CB	CB	-	2	-	-	7.395	61.663
CB	CB	-	5	-	-	28.995	34.874
CB	CB	-	-	1	-	14.161	48.072
CB	CB	-	-	2	-	19.774	41.019
CB	CB	-	-	5	-	25.740	35.515
CB	CB	-	-	-	1	10.914	55.759
CB	CB	-	-	-	2	9.693	57.316
CB	CB	-	-	-	5	22.288	38.843
CE	CE	1	-	-	-	32.530	35.763
CE	CE	2	-	-	-	66.797	21.675
CE	CE	5	-	-	-	83.739	16.724
CE	CE	-	1	-	-	12.553	55.443
CE	CE	-	2	-	-	8.311	64.993
CE	CE	-	5	-	-	7.086	65.839

"Table 4.2 : Continued…"

Formulation						Parameters	
R_t	T_t						
Base		Polymer amount (% w/w)					
		CBP	HPMC	PVP	CMCTS	f_1	f_2
CE	CE	-	-	1	-	4.438	75.840
CE	CE	-	-	2	-	4.318	74.878
CE	CE	-	-	5	-	3.883	80.308
CE	CE	-	-	-	1	5.691	71.193
CE	CE	-	-	-	2	13.039	54.323
CE	CE	-	-	-	5	2.362	83.401
SS	SS	1	-	-	-	38.049	29.091
SS	SS	2	-	-	-	71.912	15.819
SS	SS	5	-	-	-	89.125	11.003
SS	SS	-	1	-	-	3.873	76.739
SS	SS	-	2	-	-	3.269	79.135
SS	SS	-	5	-	-	5.233	70.184
SS	SS	-	-	1	-	5.967	67.582
SS	SS	-	-	2	-	5.956	66.663
SS	SS	-	-	5	-	6.636	65.966
SS	SS	-	-	-	1	10.454	57.512
SS	SS	-	-	-	2	6.524	68.781
SS	SS	-	-	-	5	3.937	78.985

4.3.3.1.2 Dissolution efficiency (DE)

The DE (Figure 4.3) provided an insight on the amount of DcNa released over a time period, reflecting the extent of drug release from the formulations examined. Among DcNa only formulations, CE showed a significantly lower DE compared to both CB and SS; this correlated well to the observations from Figure 4.2 where DcNa release rates were lower in CE. Statistical comparison of the formulations using Tukey's HSD analysis is tabulated in Appendices 22-24.

In general, the addition of CBP reduced extent of DcNa release in a concentration dependent manner in all three bases (Figures 4.3a–c). The DE of suppositories containing 5 %w/w CBP was decreased by 60 % in CB and 80 % in both CE and SS suppositories.

On the other hand, addition of 5 %w/w PVP resulted in a slight but significantly lower DE in CB, CE and SS suppositories. While the incorporation of 1-5 %w/w HPMC did not significantly alter the DE of CB suppositories; it resulted in a slight decrease in DE for both CE and SS suppositories. CB suppositories incorporated with 1-5 %w/w CMCTS had a significantly lower DE while CE suppositories containing 2-5 %w/w CMCTS resulted in a higher DE. The DE of CB suppositories containing 1-5 % w/w HPMC and SS suppositories containing 1-5 %w/w CMCTS remained unchanged compared to DcNa only suppositories. There were however, no consistent trends on the effects of HPMC, PVP and CMCTS on DE of all formulations.

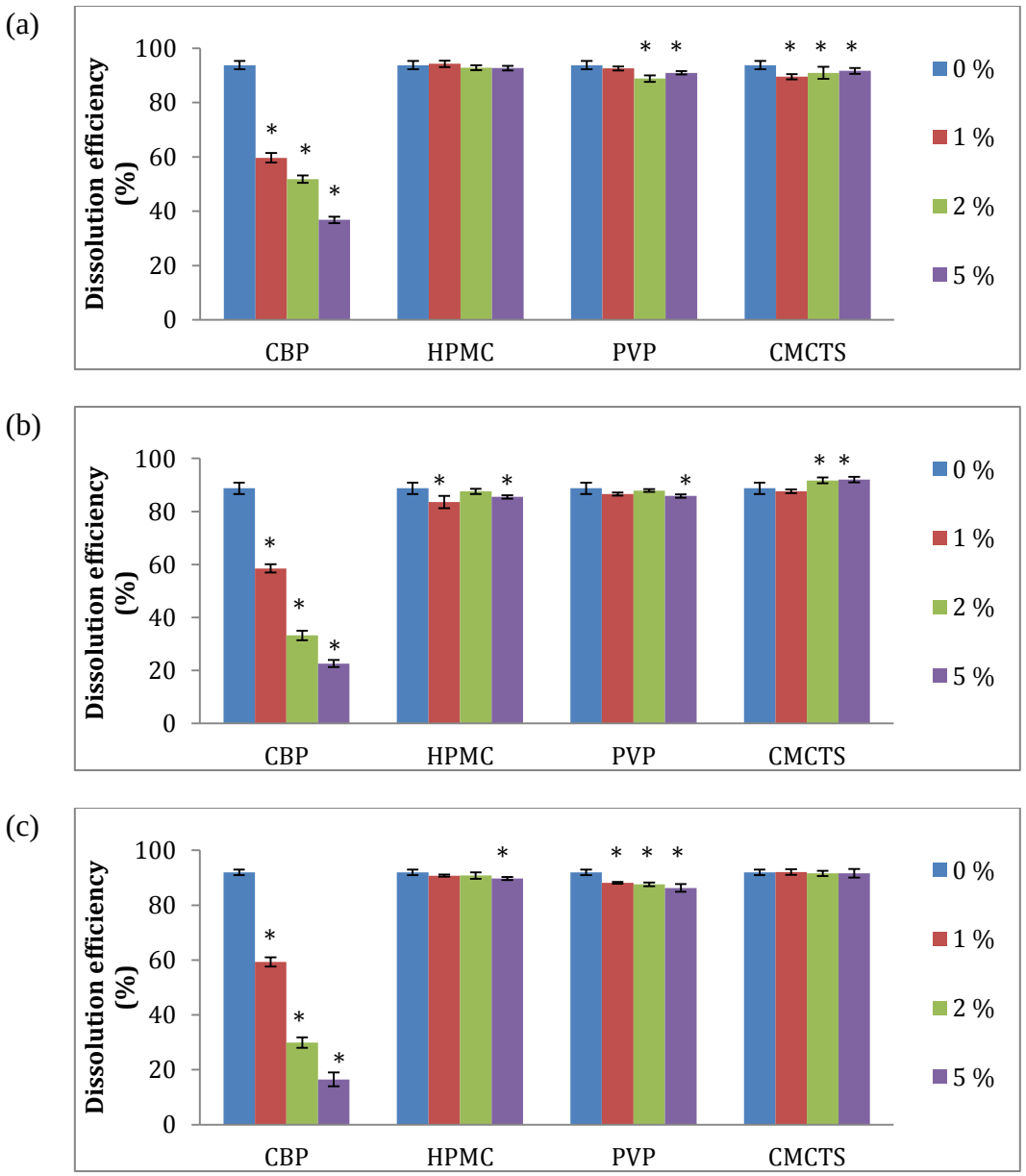


Figure 4.3 : The DE of suppository formulations containing 50 mg DcNa and 1-5%w/w bioadhesive polymers (CBP, HPMC, PVP, CMCTS) in (a) CB; (b) CE; and (c) SS. Asterisks indicate formulations which are significantly different from formulations without polymer (DcNa only). Mean ± SD, n=6.

4.3.3.1.3 Mean dissolution time (MDT)

MDT reflects amount of time required for completion of drug release. The longer the MDT, the slower the rate of DcNa release from suppositories. Figures 4.4a-c showed

the comparison of MDT generated for each formulation. Tukey's HSD post hoc test at 95 % confidence interval is tabulated in Appendices 25-27.

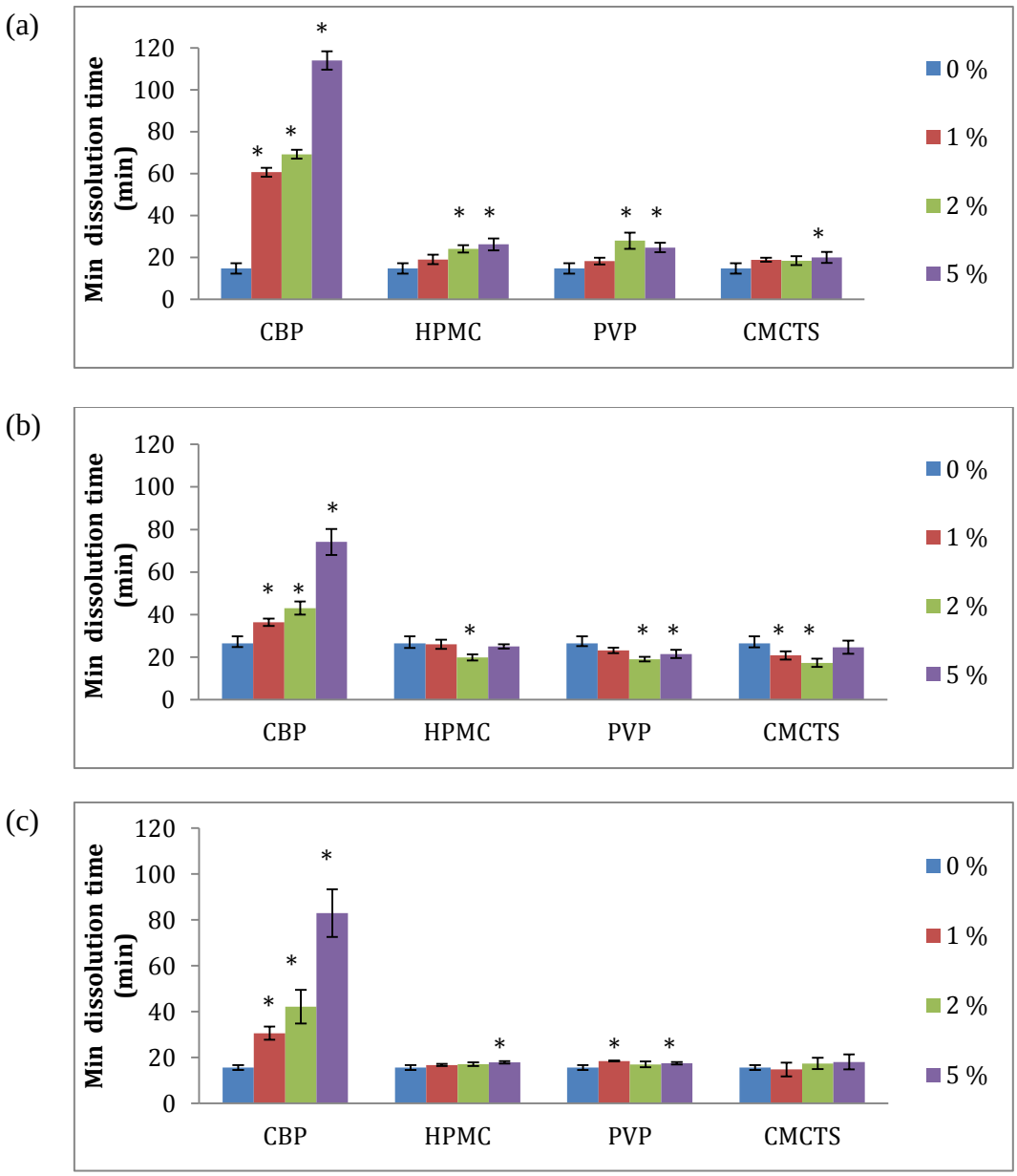


Figure 4.4 : The MDT of suppository formulations containing 50 mg DcNa and 1-5 %w/w bioadhesive polymers (CBP, HPMC, PVP, CMCTS) made from (a) CB; (b) CE; and (c) SS. Asterisks indicate formulations which are significantly different from formulations without polymer (DcNa only). Mean ± SD, n=6.

Figures 4.4a-c showed that formulations containing 1-5 %w/w CBP had significantly longer MDT compared to DcNa only formulations. This increase in MDT caused by incorporation of CBP was concentration dependent and was consistent in all three bases. The higher the CBP content, the slower the rate of DcNa release (reflected by longer MDT, Figures 4.4a-c) accompanied by a lowering in the maximum percentage of DcNa released at the end of 6 hours (reflected by DE, Figures 4.3a-c). This observation was similar to the report of impaired ramosetron release from suppositories containing 2-10 %w/w of Carbopol 934P, where the authors hypothesised the formation of a viscous CBP gel that suppressed drug release (Yahagi et al., 2000).

Decrease in DcNa release with increasing CBP content could also be due to reduction in DcNa solubility as a result of decreased receptor medium pH brought about by dispersion of CBP. CBP is acidic and produces a solution with pH 2.7–3.5 at 0.5 %w/v (Lubrizol Advanced Materials, 2009). Kincl et al. (2004) reported that solubility of DcNa at pH 5.8 can be reduced by up to 86 folds compared to at pH 8.0.

In general, the addition of HPMC, PVP and CMCTS at 1 %w/w did not significantly alter MDT of formulations in comparison to DcNa only formulations, while 2-5 %w/w of polymers resulted in small but significant changes in MDT (Figures 4.4a-c). There was however, no clear trend of concentration-dependent alteration of MDT by HPMC, PVP and CMCTS formulations.

4.3.4 Mathematical modelling

Although fit factors were effective in showing dissimilarities between profiles while DE and MDT allowed direct comparison of the rate and extent of DcNa release between profiles; release kinetics can only be explained by substituting drug release data into mathematical equations.

Release parameters for the selected mathematical model (first order, Higuchi's, Korsmeyer-Peppas and Weibull's mathematical models) after empirical fitting of each formulation were tabulated in Tables 4.3-4.5. Higuchi's equation and Korsmeyer-Peppas model were fitted with up to 60 % of the drug dissolution data in conjunction with the assumption that these two models are valid for description of cumulative drug release of 60 % (Korsmeyer et al., 1983). Meanwhile, both the first order and Weibull's model were fitted with data up to the first time point at plateau (maximum dissolution).

Table 4.3 : The goodness of fit parameters obtained from equation fitting of drug release data from all CB formulations.

Amount of Polymer (%w/w)				**First order**		**Higuchi**		**Korsmeyer- Peppas**		**Weibull model**	
CBP	**HPMC**	**PVP**	**CMCTS**	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***
0	0	0	0	0.241	118.946	0.797	28.180	0.953	26.289	0.924	43.695
1	0	0	0	0.272	84.845	-1.739	100.043	0.647	79.800	0.988	50.021
2	0	0	0	0.309	126.062	-0.8502	105.469	0.720	83.61	0.938	81.581
5	0	0	0	0.3	150.781	0.728	109.206	0.892	119.661	0.872	109.000
0	1	0	0	0.49	71.925	0.876	33.536	0.943	35.357	0.870	22.522
0	2	0	0	0.532	74.000	0.779	35.813	0.956	34.117	0.862	10.463
0	5	0	0	0.621	72.642	0.842	32.951	0.985	26.452	0.792	24.267
0	0	1	0	0.51	71.557	0.875	33.067	0.946	34.616	0.870	22.428
0	0	2	0	0.611	68.686	0.908	28.720	0.952	30.523	0.841	34.969
0	0	5	0	0.558	73.484	0.799	34.870	0.967	31.623	0.851	12.567
0	0	0	1	0.393	82.570	0.674	29.759	0.977	24.096	0.927	41.995
0	0	0	2	0.418	82.956	0.745	28.414	0.978	22.881	0.901	33.132
0	0	0	5	0.523	76.730	0.681	38.436	0.960	35.066	0.923	48.734

Table 4.4 : The goodness of fit parameters obtained from equation fitting of drug release data from all CE formulations.

Amount of Polymer (%w/w)				**First order**		**Higuchi**		**Korsmeyer- Peppas**		**Weibull model**	
CBP	**HPMC**	**PVP**	**CMCTS**	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***
0	0	0	0	0.306	118.806	0.765	27.229	0.966	23.620	0.946	54.683
1	0	0	0	0.212	144.910	-0.886	108.831	0.675	94.124	0.983	91.430
2	0	0	0	0.226	137.598	-1.003	151.483	0.692	133.769	0.937	104.763
5	0	0	0	0.319	142.782	0.827	112.236	0.824	134.667	0.859	111.005
0	1	0	0	0.438	76.189	0.522	39.593	0.915	40.066	0.948	39.330
0	2	0	0	0.411	74.169	0.731	37.803	0.903	40.125	0.947	34.930
0	5	0	0	0.494	72.340	0.775	35.636	0.943	35.743	0.908	33.777
0	0	1	0	0.476	80.340	0.883	32.101	0.950	33.517	0.890	35.355
0	0	2	0	0.369	83.874	0.731	37.803	0.957	40.125	0.950	42.744
0	0	5	0	0.382	82.377	0.757	36.793	0.903	39.416	0.953	41.415
0	0	0	1	0.377	84.159	0.687	38.283	0.957	40.693	0.954	37.455
0	0	0	2	0.430	74.676	0.590	30.996	0.970	26.301	0.899	35.078
0	0	0	5	0.417	81.403	0.777	35.864	0.910	38.400	0.935	39.156

Table 4.5 : The goodness of fit parameters obtained from equation fitting of drug release data from all SS formulations.

Amount of Polymer (%w/w)				**First order**		**Higuchi**		**Korsmeyer- Peppas**		**Weibull model**	
CBP	**HPMC**	**PVP**	**CMCTS**	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***	r^2	***AIC***
0	0	0	0	0.229	119.01	0.729	29.433	0.952	27.754	0.938	61.568
1	0	0	0	0.213	143.137	-6.839	183.66	0.643	133.642	0.986	87.630
2	0	0	0	0.183	132.781	-1.748	150.488	0.616	131.717	0.932	110.567
5	0	0	0	0.291	125.585	0.554	110.627	0.798	117.730	0.926	91.910
0	1	0	0	0.439	67.757	0.834	26.59	0.934	26.488	0.922	36.212
0	2	0	0	0.401	68.72	0.786	28.116	0.926	27.885	0.948	35.007
0	5	0	0	0.459	69.178	0.810	26.985	0.958	24.801	0.919	29.679
0	0	1	0	0.421	75.566	0.857	25.833	0.923	26.627	0.895	47.908
0	0	2	0	0.408	75.584	0.851	25.974	0.916	26.955	0.925	41.593
0	0	5	0	0.409	75.025	0.868	25.225	0.924	26.167	0.945	33.686
0	0	0	1	0.345	71.832	0.763	29.905	0.966	26.243	0.953	44.333
0	0	0	2	0.377	70.348	0.815	28.102	0.962	25.600	0.952	38.441
0	0	0	5	0.374	70.717	0.771	28.900	0.949	27.092	0.962	33.666

The formulations containing only DcNa (no bioadhesive polymers) displayed the best 'goodness of fit' to the Korsmeyer-Peppas model (r^2> 0.95), supported by the AIC values. The order of increasing possibilities of fit to the kinetic equations based on AIC values were first order (lowest), Weibull's model, Higuchi's equation, Korsmeyer-Peppas (highest).

The n-values obtained through fitting of data into the Korsmeyer-Peppas equation (Table 4.6) were within the range of 0.45–1.00, which was indicative of non-Fickian diffusion of DcNa from the suppositories.

In general, the incorporation of HPMC, PVP and CMCTS did not affect the release mechanism of DcNa from the suppositories. The DcNa release was predominantly via non-Fickian diffusional methods as the Korsmeyer-Peppas release exponent (n), ranged between 0.580–0.980 (Table 4.6). DcNa release from the suppositories were therefore diffusion and erosion-controlled. These polymers (HPMC, PVP and CMCTS) dissolve upon contact with dissolution media to form gaps which facilitates erosion of the suppository matrix while DcNa simultaneously diffuse out of the DcNa-drug matrix.

Table 4.6 : The release constant (k_m) and release exponent (n) of formulations fitted to Korsmeyer- Peppas model.

Formulation				**Korsmeyer –Peppas parameters**	
Base	**Amount of Polymer (%w/w)**				
CB	**HPMC**	**PVP**	**CMCTS**	k_m	n
	0	0	0	7.945	0.878
	1	0	0	7.772	0.756
	2	0	0	4.382	0.896
	5	0	0	5.157	0.795
	0	1	0	7.285	0.759
	0	2	0	8.096	0.584
	0	5	0	6.569	0.662
	0	0	1	4.489	0.949
	0	0	2	5.649	0.953
	0	0	5	3.228	0.980
CE	0	0	0	5.539	0.931
	1	0	0	7.109	0.802
	2	0	0	7.704	0.642
	5	0	0	4.366	0.898
	0	1	0	7.080	0.747
	0	2	0	4.370	0.957
	0	5	0	4.770	0.915
	0	0	1	3.589	0.913
	0	0	2	3.460	0.916
	0	0	5	4.992	0.882
SS	0	0	0	6.221	0.972
	1	0	0	9.442	0.792
	2	0	0	8.287	0.860
	5	0	0	7.390	0.862
	0	1	0	7.668	0.895
	0	2	0	8.073	0.878
	0	5	0	8.179	0.862
	0	0	1	7.644	0.934
	0	0	2	8.714	0.856
	0	0	5	7.405	0.914

Meanwhile, addition of 1-5 %w/w CBP resulted in a change of DcNa release mechanism in CB, CE and SS where poor fit of the data to Korsmeyers–Peppas model ($r^2 < 0.9$) was observed (Tables 4.3-4.5). Instead, these data was better fitted to the Weibull's model (Table 4.7) which describes release of matrix-like drugs (higher R^2 and smaller AIC). This could be due to the higher viscosity of molten suppository mixture (in the presence of DcNa and CBP) coupled with gelling properties of CBP upon contact with water which subsequently lead to trapping of DcNa within a base-DcNa matrix.

Based on the Weibull's model, Table 4.7 showed that as the concentration of CBP increased, the scale factor (α) which corresponded to apparent rate constant decreased. This was in good agreement with the findings in Sections 4.3.3.1.2 and 4.3.3.1.3 that showed prolonged MDT and lowered DE as the concentration of CBP increased.

On the other hand, the shape dependence factor (β) were within the range of 0.3–0.7 (<1), which described the shape of drug release curves as having a steeper initial slope than exponential release curves. The β value of the formulations containing 1-5 %w/w CBP in this study reflected drug release in accordance to Fickian's diffusion ($\beta < 0.75$), as quoted by Papadopoulou et al. (2006). As the suppository melts and CBP starts to gel in contact with dissolution medium, it traps DcNa within the matrix. As gelling continues, the gel layer forms a barrier which impedes DcNa release, similar to that observed in drug release for devices with fractal geometry (Kosmidis et al., 2003). DcNa release from the dosage form is therefore expected to be proportionate to the fraction of particles that are sufficiently close to the barrier surface to diffuse from the

dosage form, down its concentration gradient (Kosmidis et al., 2003; Papadopoulou et al., 2006).

Table 4.7 : The release parameters of formulations containing CBP fitted with Weibull equation.

Formulation		**Weibull model parameters**	
Base	**CBP (%w/w)**	**α (time dependence factor)**	**β (shape dependence factor)**
CB	**1**	15.216	0.297
	2	10.702	0.334
	5	0.417	0.754
CE	**1**	10.321	0.370
	2	3.779	0.404
	5	0.795	0.620
SS	**1**	16.605	0.239
	2	3.636	0.391
	5	0.916	0.534

Despite a generally better fit of formulations containing CBP to the Weibull model (smaller AIC), the goodness of fit in terms (r^2) of the formulations at higher concentrations of CBP were still < 0.95 (Tables 4.3-4.5). Thus, further investigation using a biphasic release model (bi-exponential first-order kinetic model) was attempted. Parameters obtained from fitting into bi-exponential first-order kinetic equation were described in Table 4.8.

Table 4.8 : The release parameters of formulations containing 1-5 %w/w CBP fitted with bi-exponential first-order kinetic equation. All the r^2 values for initial and terminal phases were > 0.95 when goodness of fit of the data was reviewed by linear regression.

Formulation		**Bi-exponential first-order kinetics parameters**				
Base	**CBP (%w/w)**	**A**	***k_1*** **(min^{-1})**	**B**	***k_2*** **(min^{-1})**	**Lag time (min)**
CB	**1**	49.422	0.282	54.495	0.002	0.290
	2	42.423	0.200	61.271	0.001	0.451
	5	28.968	0.026	74.344	0.001	4.185
CE	**1**	52.305	0.217	56.821	0.002	0.870
	2	27.975	0.102	75.384	0.001	1.205
	5	21.313	0.057	82.914	0.001	3.701
SS	**1**	49.979	0.247	58.096	0.004	0.699
	2	28.011	0.108	74.911	0.001	1.000
	5	11.642	0.062	89.407	0.001	1.427

The CBP formulations showed good linearity and reasonable lag times with bi-exponential first-order kinetic equation. The results suggested that DcNa was released from suppositories via a rapid initial phase (k_1) followed by a slow terminal phase drug release (k_2). The addition of CBP increased lag times and decreased k_1 in a concentration dependent manner.

The bi-exponential first-order kinetic fitting was initially modelled to characterise rapid intravenous injections. Over time, it has been used to describe the release of

drugs from solid dosage forms such as tablets and capsules (Laakso et al., 1984). In tablets, the rapid initial release was described as a result of increasing surface area for dissolution following tablet disintegration while the slower phase describes diffusion of drug from the dosage form.

This concept can be adapted to explain DcNa release form CBP suppositories. During dissolution, the suppository undergoes initial melting followed by formation of fatty globules (containing molten base, DcNa and CBP). At this stage, DcNa release is via both diffusion from the molten base as well as erosion or deformation of the dosage form during the melting process (k_1). But as CBP comes in contact with the dissolution medium, it starts to gel and eventually form a barrier between the matrix (base with DcNa) and dissolution medium, whereby DcNa can now only be released via a slow diffusion process across the barrier (k_2). Weibull's equation provided information on possible mechanism of DcNa release from CBP suppositories while the bi-exponential first-order equation described the release rate and kinetics during the biphasic release process; thus both models were used concomitantly to describe the release of DcNa from CBP suppositories.

4.4 Conclusion

The HPKS bases were comparable to CB in terms of drug release capacity and could be good lipophilic base candidates for fast-acting DcNa suppository formulations.

Although convenient, the exploratory data analysis methods were unable to compare between large number of drug release profiles and offered no explanations to drug release mechanisms. The fit factors allowed quick detection of dissimilar profiles yet

does not identify cause of the differences between profiles. DE and MDT were useful for comparing differences between rate and extent of drug release but only mathematical modelling enabled the prediction of DcNa release mechanism. However, none of these methods were sufficient as a standalone analysis and thus, they should be used concomitantly to provide a complete picture of drug release profiles.

Generally, the model independent methods (fit factors, DE, MDT) provided strong indication that DcNa release from formulations containing 1-5 %w/w CBP was markedly different (statistically significant at 95 % confidence interval) from their respective reference formulations (DcNa only suppositories). Although the fit factors found that 5 %w/w PVP, HPMC and CMCTS made in CB were significantly different from reference formulations (containing only DcNa), this difference was mainly reflected in terms of DE rather than due to a change in mechanism of drug release.

Mathematical modelling of data found that suppositories containing only DcNa released the drug via non-Fickian diffusion kinetics. Addition of 1-5 %w/w HPMC, PVP and CMCTS to the formulations did not alter mechanism of DcNa release. They are therefore suitable candidates of bioadhesive polymers for development of DcNa suppositories.

However, the addition of CBP lead to considerable change in morphology of molten suppository during dissolution via gelling, which resulted in biphasic DcNa release process involving a rapid initial diffusion and erosion process followed by slow diffusion process across the CBP gel layer. Furthermore, as CBP gels in the dissolution medium, it decreases the environment pH which leads to decreased DcNa

solubility, thus further retarding the release of DcNa from suppositories. The concentration dependent impedance of DcNa release from CBP suppositories indicate that CBP should only be used at the lowest possible concentration to confer bioadhesivity as concentrations of 1-5 %w/w had evidently suppressed drug release.

The drug release studies using distilled water were only preliminary in nature. Drug release studies using buffered solutions at rectal pH of 7.2 should be considered after further investigation on bioadhesive properties and enhancement on formulation prototypes.

CHAPTER 5

BIOADHESION STUDIES

5.1 Introduction

5.1.1 Methods to study bioadhesion

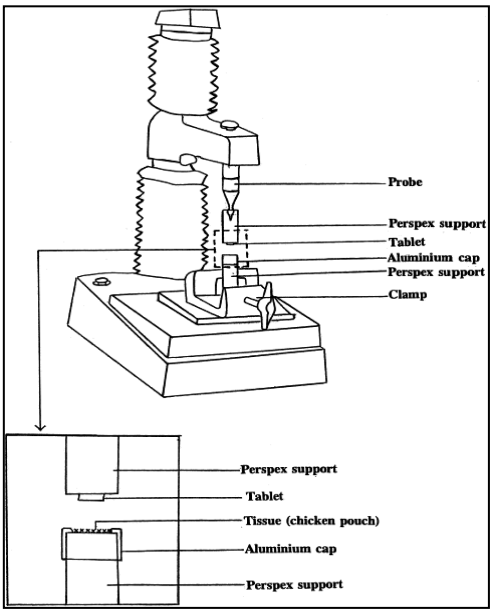
Bioadhesion studies can be performed via *in vitro*, *in vivo* and *ex vivo* experimental setups. *In vitro* bioadhesion studies are by far most commonly adopted due to ease of experimental setup. This method involves the use of a suitable excised mucosal membrane or synthetic membrane surface as the site of attachment under simulated conditions.

To date, various methods have been developed and applied to study bioadhesive properties of pharmaceutical formulations. Among the techniques employed were (a) florescence probe technique (Park and Robinson, 1984) which measures change in fluorescence upon binding of polymer to epithelial cells labelled with pyrene and fluorescein, (b) detachment stress methods including Wilhelmy plate method (Sam et al., 1992; Santos et al., 1999), tensile stress method (Smart, 1991; Thirawong et al., 2007; Tobyn et al., 1995; Wong et al., 1999a) and shear stress method (Jiménez-Castellanos et al., 1993; Leung and Robinson, 1988; Mortazavi and Smart, 1995; Wang and Tang, 2008); (c) the wash-off method (Lehr and Bouwstra, 1992) which quantifies amount of particulate remaining on the test surface after bouts of agitation; and (d) mucin-particulate method (Takeuchi et al., 2005) which measures change in zeta potential of mucin brought about by mucin-polymer interaction. The Biacore® method to study bioadhesion was subsequently developed as an extension of this concept, where the change in surface plasmon resonance brought about by interaction between polymer and mucin reacted onto chitosan-immobilised sensor chips were measured arbitrarily (Thongborisute and Takeuchi, 2008). Of these, the detachment methods were most commonly used on solid and semi-solid dosage forms.

5.1.2 Detachment methods

5.1.2.1 Tensile stress

(a)



(b)

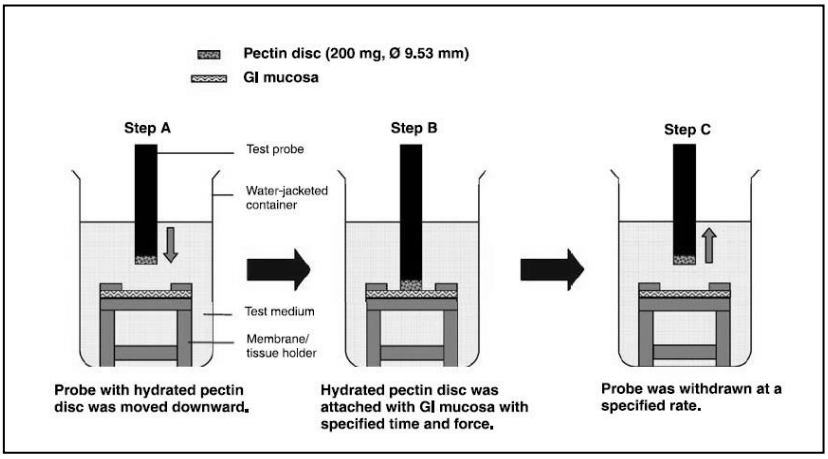


Figure 5.1 : Experimental setup for testing tensile stress of bioadhesion using texture analyser; (a) without temperature control (Thirawong et al., 2007; Tobyn et al., 1995; Wong et al., 1999a) and; (b) with temperature control (Thirawong et al., 2007).

The tensile setup measures force required to fracture bioadhesion bond at right angles to the plane of contact between test sample and mucosa surface. Wilhelmy plate method (Smart et al., 1984) measures the tensile stress generated upon detaching a glass plate coated with polymer vertically immersed in mucin using a microforce

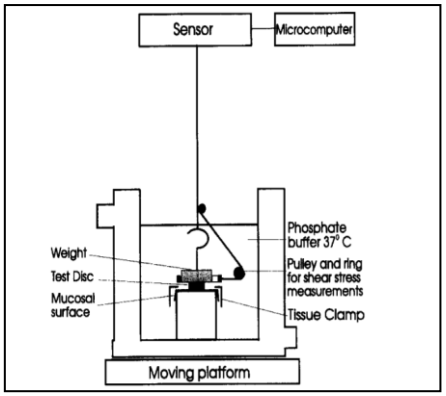
balance. This concept was subsequently modified to measure tensile stress generated upon the detachment between a tablet and mucosa surface using the tensiometer (Leung and Robinson, 1988; Ponchel et al., 1987) and texture analyser (Thirawong et al., 2007; Tobyn et al., 1995; Wong et al., 1999a). Figures 5.1a-b shows some of the experimental setup developed by other researchers to measure tensile stress of force required to detach dosage form from the mucosa.

5.1.2.2 Shear stress

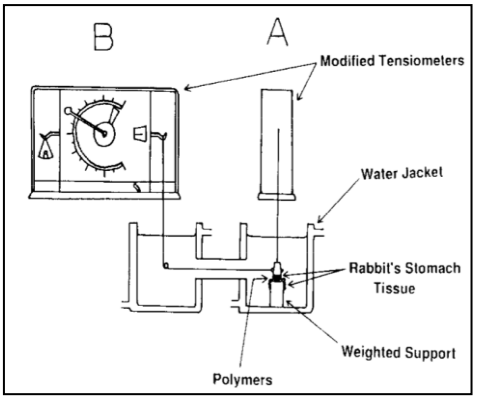
Figure 5.2 shows some of the experimental setups developed to measure tangential shear stress. Shear setup measures the sliding force parallel to the plane of contact required to dislodge a sample disc from mucosa surface.

Most studies measure the degree of bioadhesion in terms of tensile stress or tensile strength, although in actual fact, dosage forms administered into the GIT or vagina or buccal regions are most likely to undergo frictional and shear stress which occurs parallel to the adhesive joint. This might be due to the difficulties in measuring shear stress as well as the inadequacy of current methods to distinguish between actual force from the bond joint fracture and the force contributed by the friction of both surfaces (Jiménez-Castellanos et al., 1993; Leung and Robinson, 1988; Mortazavi and Smart, 1995). While Leung and Robinson (1988) reported good results with their experimental setup (Figure 5.2b), Mortazavi and Smart (1995) were unable to yield comprehendible shear stress readings using the setup in Figure 5.2a because the readings were affected by friction and the occurrence of re-adhesion after joint fracture under the influence of gravity.

(a)



(b)



(c)

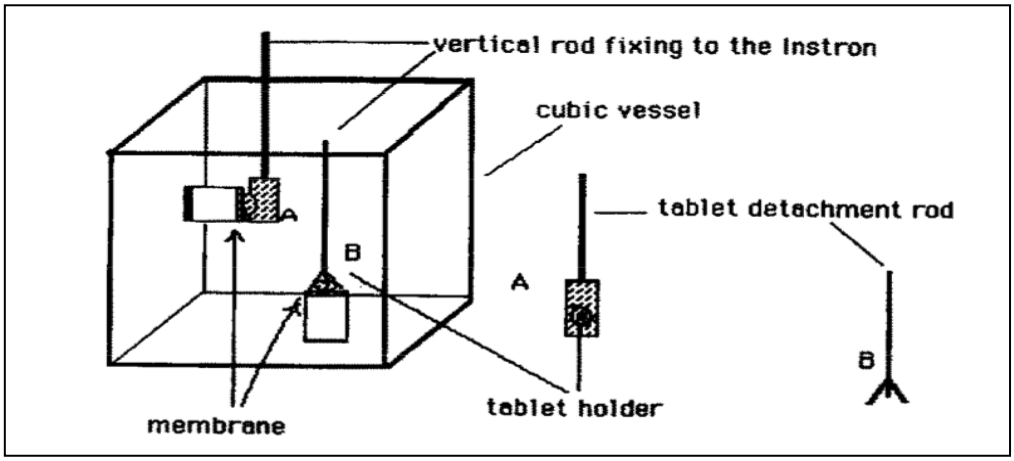


Figure 5.2 : Experimental setups used to investigate the shear stress of the bioadhesion joint between bioadhesive material and membrane; (a) modified Dia-Stron rheometer equipped with pulley system (Mortazavi and Smart, 1995); (b) dual modified tensiometer method (Leung and Robinson, 1988) and; (c) vertical rod coupled to tensiometer (Jiménez-Castellanos et al., 1993).

5.1.3 Experimental design considerations

Methods to measure stress of detachment vary greatly in terms of experimental setup, choice of mucosa membrane and test medium used, in order to simulate *in vivo* conditions at which bioadhesion is expected to take place.

Experimental setup designed for testing bioadhesion in the GIT involved complete immersion of tissue and dosage form samples in a suitable test medium (Figure 5.1b and Figures 5.2a-b); usually simulated gastric which served as both a hydration medium and water bath to maintain the tissue at physiological temperature (Thirawong et al., 2007; Tobyn et al., 1995). This however, may not be applicable for the testing of bioadhesive suppositories as the rectum contains only 2–3 mL of inert mucus over a surface area of 200–400 cm^2 (Allen et al., 2008). Conversely, some studies excluded immersion of tissue in test medium, but at the expense of physiological temperature control (Figure 5.1a and Figure 5.2c) (Jiménez-Castellanos et al., 1993; Ponchel et al., 1987; Wong et al., 1999a). Temperature control was particularly important for current work as suppositories would soften and melt at human body temperature, yet immersion of samples in test medium does not reflect physiological rectal conditions.

Bioadhesive polymers were incorporated into rectal suppositories in this research as an attempt to circumvent the pre-systemic first-pass metabolism. It is hoped that the bioadhesive polymers would enable adherence of suppositories to the rectal mucosa, thus preventing its movement towards the upper rectum where capillaries drain into the hepatic portal system which is responsible for a substantial degree of pre-systemic drug inactivation. Previous studies by Yahagi et al. (2000) and Ramadan (2012) have incorporated CBP into suppositories, however, bioadhesive properties of the formulations were not tested.

Therefore, this chapter aims to develop and optimise methods for *in vitro* assessment of bioadhesivity of suppositories using the texture analyser for measurement of tensile

and shear stresses required to disrupt bioadhesion. Measurements were made in terms of peak force of detachment (F_{max}) and work of adhesion (W_{ad}). This is followed by evaluation of bioadhesive properties of the suppository formulations developed in Chapter 3. Finally, as efforts to diversify experimental setup, synthetic cellulose membrane was investigated as a potential alternative to biological membranes in bioadhesion studies.

5.2 Materials and methods

5.2.1 Materials

Type III mucin from porcine stomach was purchased from Sigma Aldrich, Missouri, USA. The regenerated cellulose membrane (nominal MW 12,000–14,000; thickness 33 mm) was purchased from Fisher Scientific. Other materials used have been described in Sections 2.2 and 3.2. All reagents were analytical grade. The materials were used as received.

5.2.2 Methods

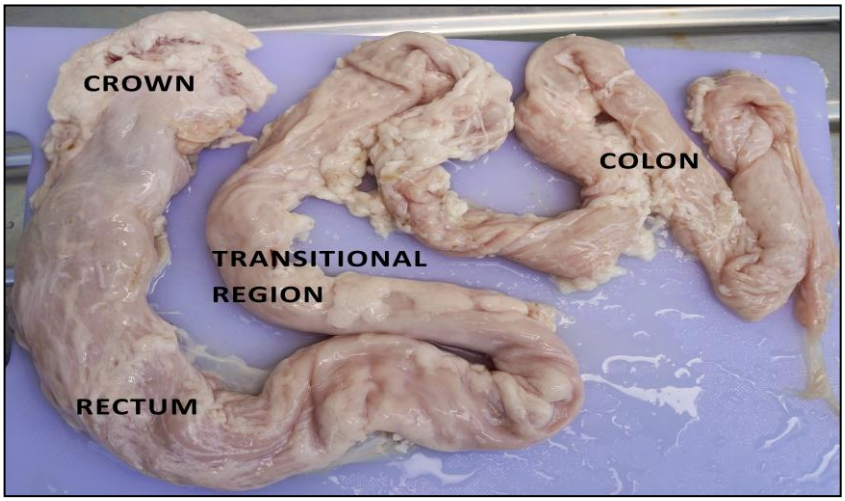
5.2.2.1 Preparation of sample discs

Cylindrical sample discs with a radius of 1.3 cm and thickness of 0.5 cm were prepared via fusion moulding. Method of manufacturing was similar to that of suppositories (Section 3.3.1) with the exception of acrylate disc moulds in place of suppository moulds. Bioadhesive polymers (CBP, HPMC, PVP and CMCTS) were added to the molten base at concentrations of 1, 2 and 5 %w/w alongside 50 mg of DcNa per disc.

5.2.2.2 Preparation of large intestinal tissue

Freshly excised porcine large intestines were obtained from a local slaughterhouse and processed within 24 hours. The large intestines were split lengthwise and luminal contents were removed by careful rinsing with distilled water. The serosa, tunica muscularis and the submucosa layer were removed and the large intestines were separated into three sections - the crown, rectum and colon (Figures 5.3a-b).

(a)



(b)

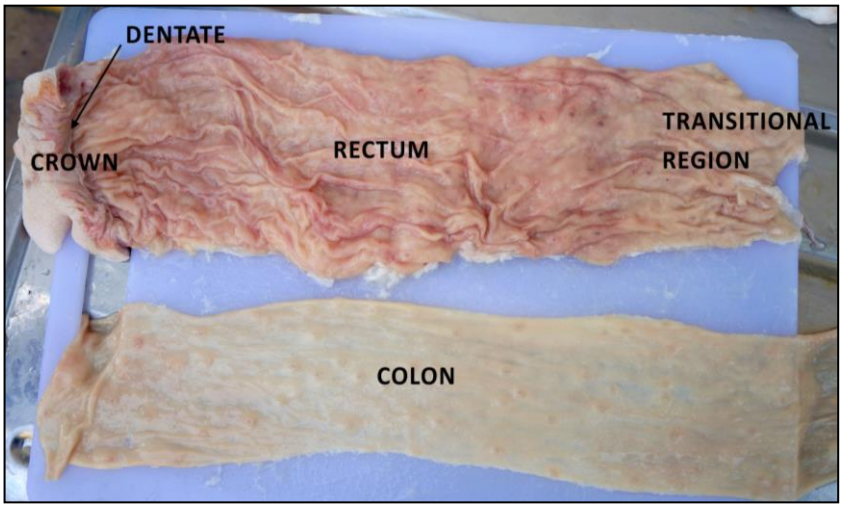


Figure 5.3: Different segments of freshly excised porcine large intestinal tissues used in this study, (a) intact large intestines with serosa, tunica muscularis and submucosa layer; (b) split large intestines, with mucosa facing upwards.

The crown was discarded and rectum region was harvested 3 cm after the dentate while 10-15 cm of the mucosa between rectum and colon were discarded to omit 'transitional regions'. The rectum and colon were carefully examined to ensure the membrane is intact before cutting into 6 cm x 6 cm membrane segments. The membranes were immersed in 0.9 %w/v sodium chloride and kept frozen until use, within 1 week from the day of processing. The yield of sample membranes from the intestines was tabulated.

5.2.2.3 Preparation of simulated rectal mucus

Simulated rectal mucus (SRM) was prepared by stirring 5 %w/w type III mucin in pH 7.4 simulated colonic fluid (SCF) for 3 hours. The SCF was prepared based on the formula for SCF by Marques et al. (2011). SRM was stored refrigerated at 4 °C and used within 72 hours from time of preparation.

5.2.2.4 Experimental setup

Bioadhesion measurement was conducted using a Ta.XT plus Texture Analyser (Stable Microsystems, Surrey, UK) equipped with a 5 kg load cell. All measurements were conducted at 29 ± 1 °C with RH of 55–65 %. All studies were carried out in 5–6 replicates. Fresh tissue and sample disc was used for each replicate.

5.2.2.4.1 Tensile measurement

The method used in the current study was modified from Thirawong et al. (2007) and Wong et al. (1999) to allow temperature control of the membrane without immersion of the setup in a water bath. The setup comprised of a probe affixed to the texture analyser arm with a flat round surface and a copper membrane stage heated using a

ceramic top stirring hotplate (Fisher Scientific, Pittsburg, USA). The entire assembly is as depicted in Figure 5.4.

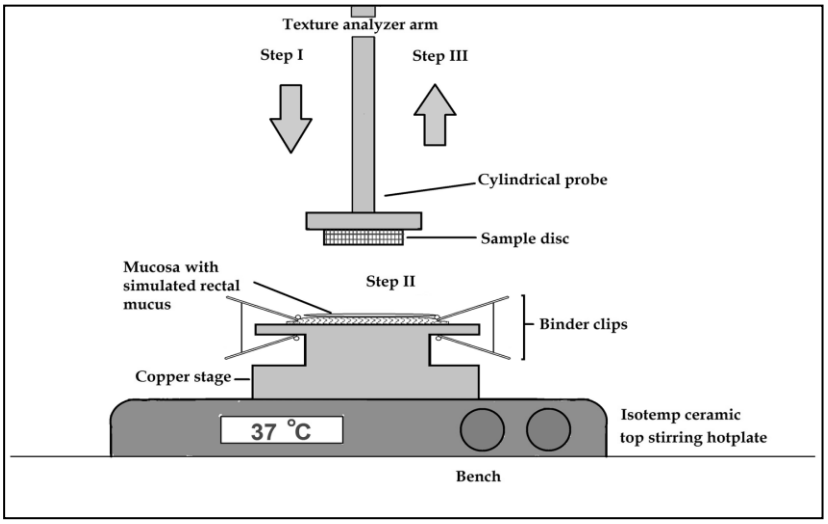


Figure 5.4 : The tensile bioadhesion study experimental setup using texture analyser with heated copper membrane stage.

Sample discs were securely mounted onto the flat surface of the cylindrical probe using double sided tape. The tissue prepared using methods describe in Section 5.2.2.2 were allowed to thaw to room temperature and immersed in SCF for 30 minutes before clamping on the copper stage using binder clips, luminal surface facing upwards. The copper stage was then positioned below the texture analyser arm and aligned to ensure the sample disc comes into direct contact with membrane surface when probe is lowered. A fixed volume of SRM was dispensed onto the mucosa surface and spread out evenly before lowering the sample disc to 5 cm above the membrane. The membrane temperature was measured using an infrared thermometer and allowed to equilibrate to 37.0 ± 0.5 °C on the copper stage before commencement of experiments. The probe was lowered at a speed of 1 mm/s until contact was made

between the sample disc and the membrane (Figure 5.4, Step I). This contact was maintained for a specific time (contact time) under a fixed contact force (Figure 5.4, Step II). At the end of contact time, the probe was withdrawn at a predetermined speed (probe withdrawal speed) to a 10 mm distance (Figure 5.4, Step III).

5.2.2.4.2 Shear measurement

The method used in the current study was modified from Chary et al. (1999) and Wang and Tang (2008) to allow direct measurement of shear force required to disrupt bioadhesion between sample disc and the membrane under temperature control. The entire assembly is as depicted in Figure 5.5.

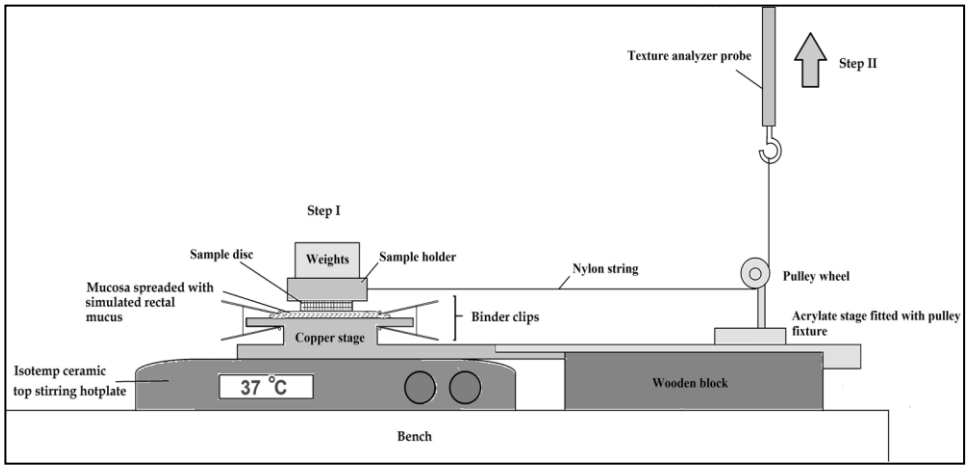


Figure 5.5 : The shear bioadhesion study experimental setup using texture analyser with heated copper membrane stage.

The setup comprised of three components: a sample holder; a copper stage connected to an acrylate stage affixed with a pulley wheel and; a texture analyser probe fitted with a hook. Sample discs were securely mounted onto the flat surface of the sample holder using double sided tape while the mucosa was attached to the copper stage

using binder clips (luminal surface upwards). The entire copper stage was maintained at 37 °C using a ceramic top stirring hotplate (Fisher Scientific, Pittsburg, USA). A fixed length of nylon string was attached from the sample holder to the hook on the probe through a pulley at right angles. The position of the probe was adjusted to ensure there is no slack along the length of the string. The membrane temperature was allowed to equilibrate to 37.0 ± 0.5 °C on the copper stage and dispensed with a fixed volume of SRM before commencement of experiments. Weights (contact force) were placed on the sample holder for a predetermined duration of time (contact time) to facilitate bioadhesion between sample disc and membrane (Figure 5.5, Step I). At the end of the contact time, the probe was withdrawn at a predetermined speed (probe withdrawal speed) to dislodge sample disc from membrane surface (Figure 5.5, Step II).

5.2.2.5 Effects of instrument and test variables on the bioadhesive test

F_{max} of the sample discs from the porcine large intestinal mucosa under different test conditions was measured. Three instrumental variables were studied; the contact time, probe withdrawal speed and contact force; while the two test variables studied were the volume of mucin used and the type of large intestinal mucosa used (rectum or colon). Studies on the instrument variables were conducted using the porcine colon mucosa with 150 μL of SRM evenly spread over the surface. All studies were carried out in 5–6 replicates.

5.2.2.5.1 Tensile measurement

Sample discs containing 50 mg DcNa and 5 %w/w CBP were used to optimise the instrument and test variables used in tensile measurements. Four different contact

times, five probe withdrawal speed, five contact forces, five volumes of SRM and two types of mucosa. The parameters are tabulated in Table 5.1.

5.2.2.5.2 Shear measurement

Sample discs containing 50 mg DcNa and 5 %w/w PVP were used to optimise the instrument and test variables used in shear measurements. Four different contact times, four probe withdrawal speed, four contact forces, four volumes of SRM and two types of mucosa. The parameters are tabulated in Table 5.2.

5.2.2.6 Evaluation of the bioadhesive strengths in suppository formulations using biological membranes

The instrumental and experimental parameters used to evaluate bioadhesive strength of suppository sample disc were obtained from studies in Section 5.2.2.5.1 and 5.2.2.5.2. All measurements were carried out in 5-6 replicates.

5.2.2.7 Evaluation of synthetic regenerated cellulose membrane as an alternative to biological membrane

Evaluation was carried out using settings and parameters used in Section 5.2.2.6 with the substitution of synthetic regenerated cellulose membrane for porcine colon mucosa. The regenerated cellulose membrane was cut to 6 cm x 6 cm squares and immersed in SCF for 1 hour prior to use. All measurements were carried out in 5-6 replicates.

Table 5.1 : The fixed and variable parameters used for tensile force optimisation using sample discs containing 50 mg DcNa and 5 %w/w CBP.

Fixed parameter	Variable parameter				
	Contact time	Probe withdrawal speed	Contact force	Volume of SRM	Type of mucosa
Contact times	5, 10, 20, 30 s	20 s	20 s	20 s	20 s
Probe withdrawal speed	10 mm/s	1, 2, 5, 10, 20 mm/s	10 mm/s	10 mm/s	10 mm/s
Contact force	2 N	2 N	0.5, 1, 1.5, 2, 3 N	2 N	2 N
Volumes of SRM	150 µL	150 µL	150 µL	0, 50, 100, 150, 300 µL	150 µL
Type of mucosa	colon	colon	colon	colon	colon, rectum

Table 5.2 : The fixed and variable parameters used for shear force optimisation using sample discs containing 50 mg DcNa and 5 %w/w PVP.

Fixed parameter	Variable parameter				
	Contact time	Probe withdrawal speed	Contact force	Volume of SRM	Type of mucosa
Contact times	20, 40, 60, 90 s	60 s	60 s	60 s	60 s
Probe withdrawal speed	30 mm/s	5, 10, 20, 30 mm/s	30 mm/s	30 mm/s	30 mm/s
Contact force	2 N	2 N	1, 2, 3, 4 N	2 N	2 N
Volumes of SRM	150 µL	150 µL	150 µL	0, 100, 150, 300 µL	150 µL
Type of mucosa	colon	colon	colon	colon	colon, rectum

5.2.2.8 Data analysis

Figure 5.6 showed the typical plot of force versus distance data obtained through tensile measurement. The maximum force required for separation of sample disc from the membrane or F_{max} was obtained directly from the force–distance curve while the work of adhesion (W_{ad}) was calculated using area under the force–distance curve using the Texture Exponent 32 software. Bioadhesive properties of the different formulations were evaluated and compared based on these two parameters. ANOVA followed by a post hoc Tukey's HSD test was performed to examine both effects of instrument and experimental variables on bioadhesion as well as bioadhesive strength of various formulations. The statistical analyses were conducted using SPSS version 20 (SPSS Inc., USA). A statistically significant difference was observed when $p < 0.05$.

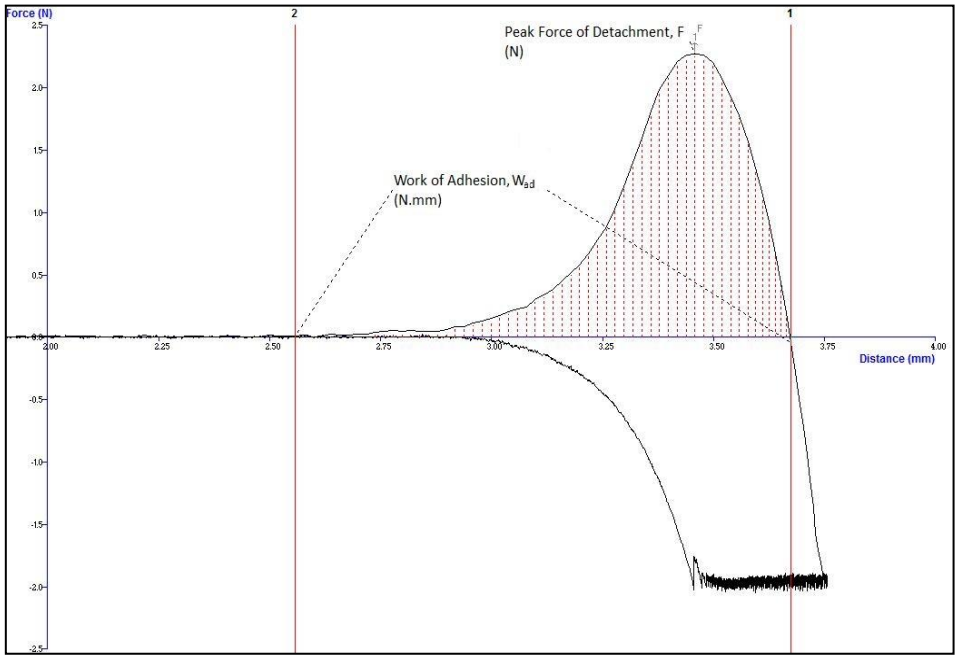


Figure 5.6: A typical plot of force versus distance data for suppository sample disc (CB + 50 mg DcNa + 1 %w/w PVP) tested with colon mucosa using the tensile setup.

5.3 Results and discussion

The design of both experimental setup for tensile and shear measurement were targeted at mimicking the internal environment of the rectum. The disc surface area was designed to reflect total surface area for bioadhesion in an actual torpedo shaped suppository with height of 2.5 cm and radius of 0.4 cm (at its barrel end). The intestinal mucosa and SRM (pH 7.4) was used to reproduce rectal environment.

5.3.1 Preparation of large intestinal tissue

The yield of both rectum and colon samples were tabulated in Table 5.3. The yield of colon membrane samples were usually 2-3 times more than the amount of rectum mucosa obtained per intestine.

Table 5.3 : The yield of biological membrane mucosa.

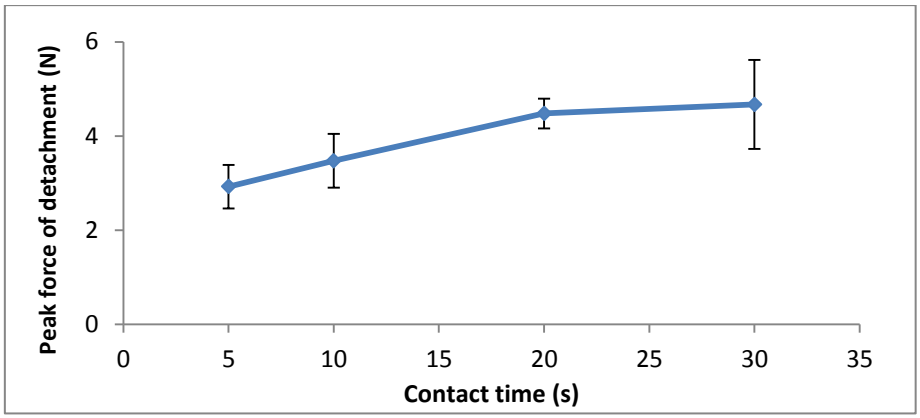
Intestine	Rectum		Colon	
	Length (cm)	Yield (6 cm x 6 cm)	Length (cm)	Yield (6 cm x 6 cm)
A	25	12	122	34
B	25	7	116	19
C	25	8	95	26
D	19	8	90	22
E	22	7	100	25
F	30	8	127	20

5.3.2 Effects of instrument and test variables on the bioadhesive test

5.3.2.1 Tensile measurement

Figure 5.7 showed that F_{max} and W_{ad} increased as contact time was increased until 20 s, where a further 10 s of contact time did not significantly increase F_{max}.

(a)



(b)

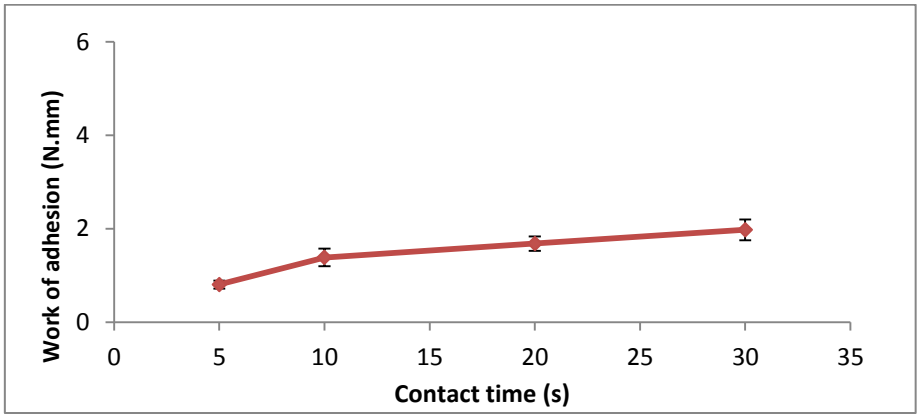
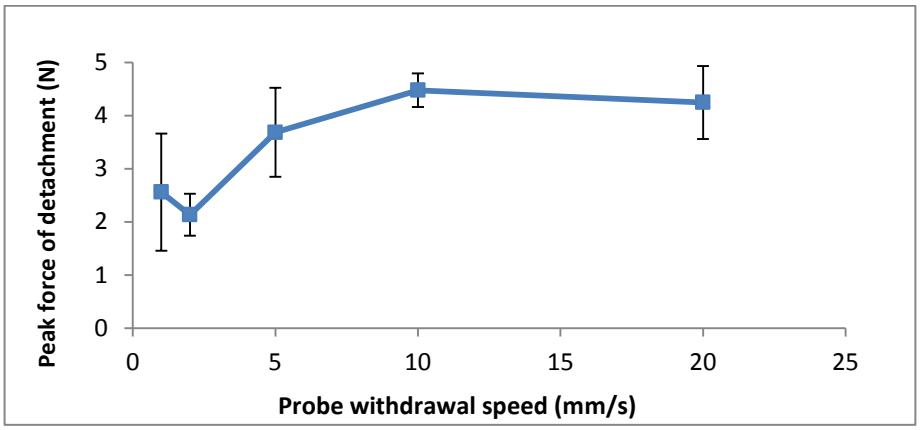


Figure 5.7: Effect of contact time on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w CBP against porcine colon mucosa using the tensile setup. Mean ± SD, n=5-6.

Increasing contact time beyond 20 s did not result in any significant increase in F_{max} and W_{ad}; contrary to previous studies by Thirawong et al. (2007) and Wong et al. (1999) which reported that increment in contact time resulted in linear increase in W_{ad}. Initial increase in contact time may have allowed interdiffusion and chain entanglement between CBP and mucin in the SRM; however as the contact time was increased, a higher fraction of the sample disc melted, resulting in an oily, slippery layer between the sample disc and the tissue mucosa. Therefore, 20 s was selected as the suitable contact time.

The effect of increasing probe withdrawal speed on both the F_{max} and W_{ad} (Figure 5.8) were similar to findings by Wong et al. (1999), and Thirawong et al. (2007). Increasing probe withdrawal speed from 1 mm/s to 10 and 20 mm/s resulted in statistically significant increase in F_{max} and W_{ad}, but there were no differences between 10 and 20 mm/s. Higher probe speeds produced larger F_{max} and W_{ad} which afforded higher sensitivities in measuring bioadhesion while the lower speeds resulted in bigger standard deviations. Therefore, an intermediate probe speed of 10 mm/s was selected for subsequent bioadhesion studies.

(a)



(b)

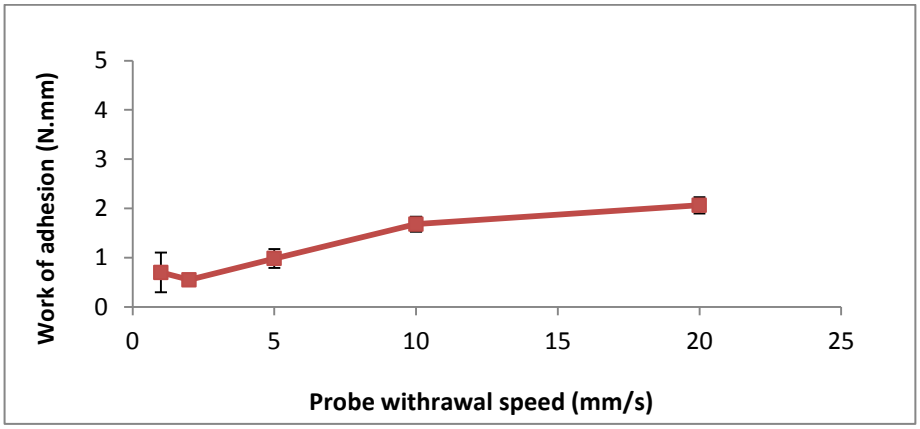


Figure 5.8: Effect of probe withdrawal speed on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w CBP against porcine colon mucosa using tensile setup. Mean ± SD, n=5-6.

A significant increase in F_{max} and W_{ad} was observed only after contact force was increased from 0.5 to 2 N (Figure 5.9). The observed trend was similar to that by Thirawong et al. (2007) although the latter study investigated contact force at the range of 0.05 to 0.5 N. Various studies have shown that basal rectal pressure is at the range of 5–25 cmH_2O (Farouk, 2003) and 20–25 mmHg (Rao et al., 1988) while anal pressure is approximately 26–75 cmH_2O (Hancock, 1976). Therefore, 2 N which translated to 0.049–0.74 N/cm^2 (equivalent to 5-75.5 cmH_2O) was selected as contact force for future studies, derived from contact surface area of sample disc (5.1 cm^2).

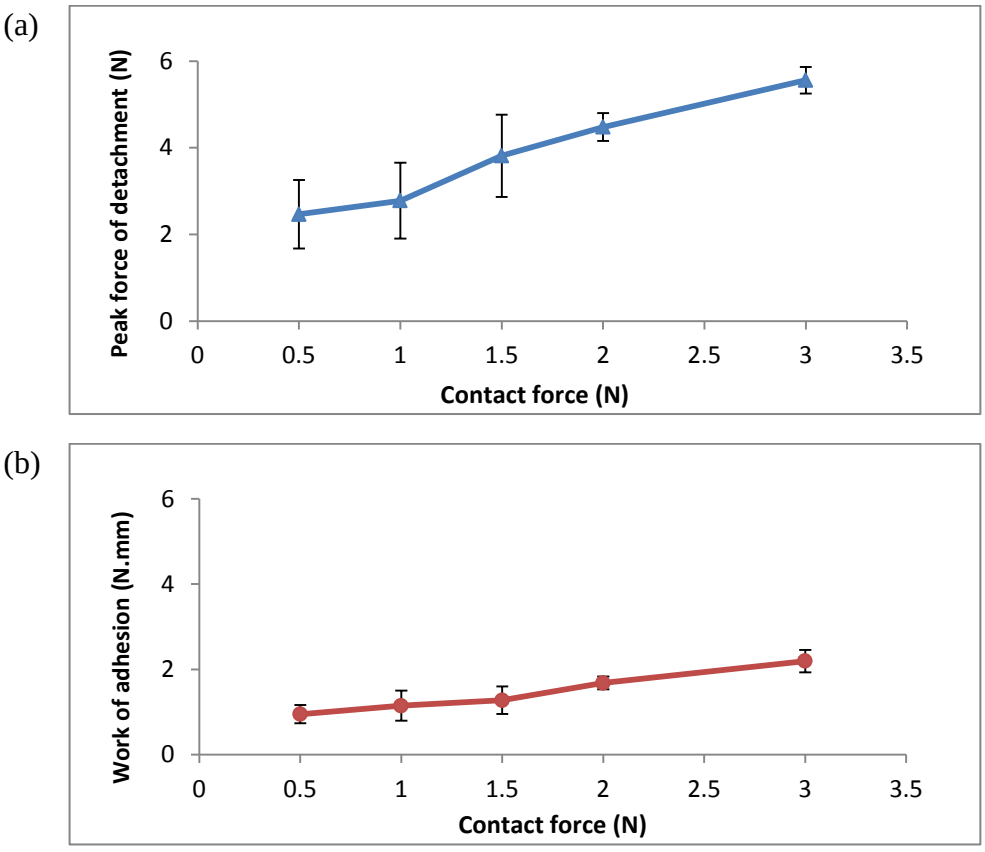
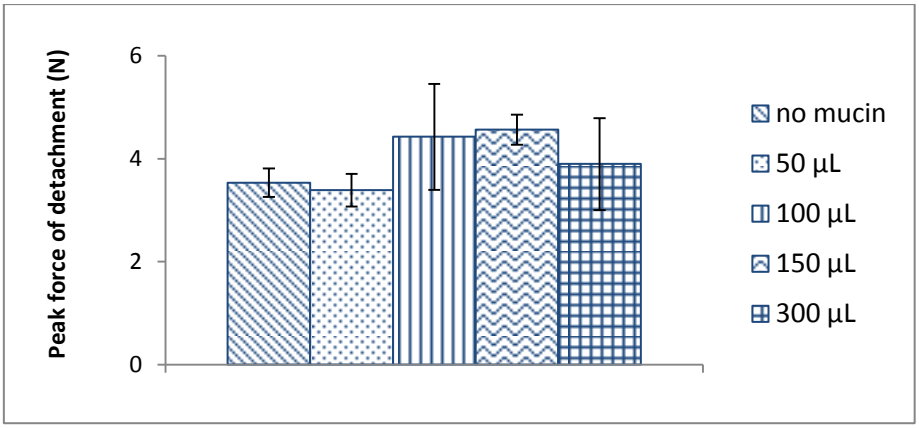


Figure 5.9 : Effect of contact force on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w CBP against porcine colon mucosa using tensile setup. Mean ± SD, n=5-6.

The effect of different SRM volumes ranging from 0–300 µL on the F_{max} and W_{ad} were investigated to select the suitable volume which simulates rectal conditions yet produced reasonable measurements of F_{max} and W_{ad}. Figure 5.10 showed that different volumes of SRM did not significantly affect the F_{max} and W_{ad} generated, although it was suggested that adhesive forces weakens as mucus content increases (Mortazavi and Smart, 1995). However, 150 µL was selected for subsequent studies as it is the average volume of rectal conditions based on the surface area of the tissue used.

(a)



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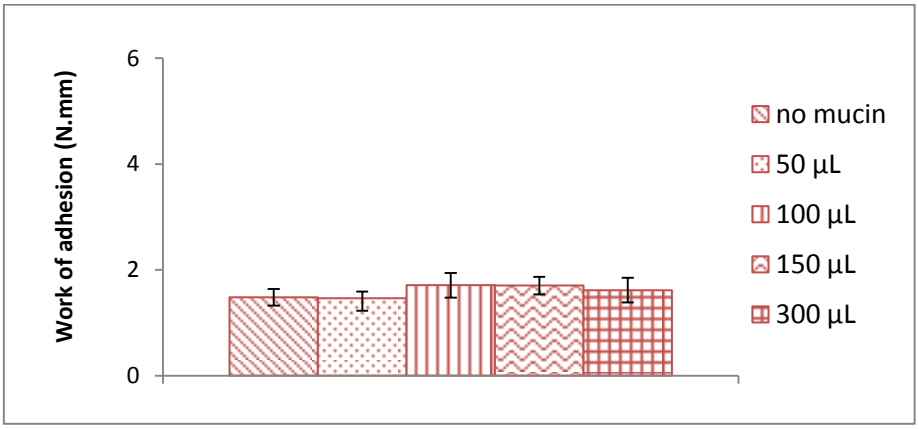
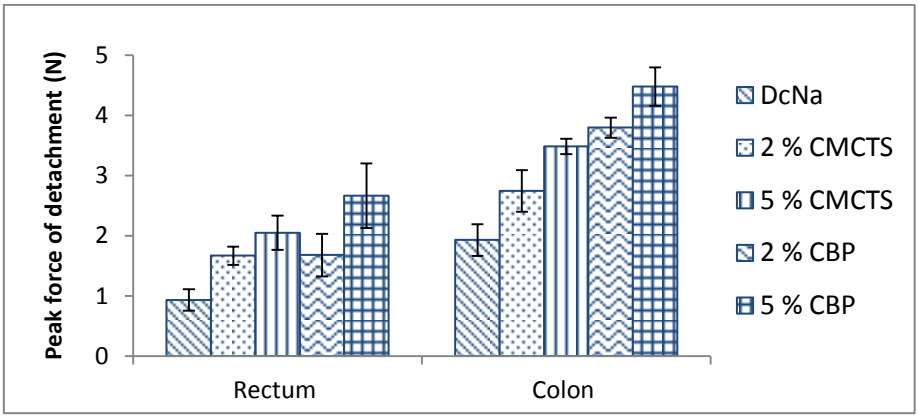


Figure 5.10 : Effect of volume of SRM on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w CBP against the porcine colon mucosa using the tensile setup. Mean ± SD, n=5-6.

This study also investigated suitability and consistency of various segments of the large intestine (rectum and colon) as model mucosa for bioadhesion studies. Generally, formulations containing either CMCTS or CBP exhibited higher F_{max} and W_{ad} values than those without polymer (Figure 5.11). Bioadhesive properties of CBP were greater than CMCTS and increased in a concentration dependant manner.

(a)



(b)

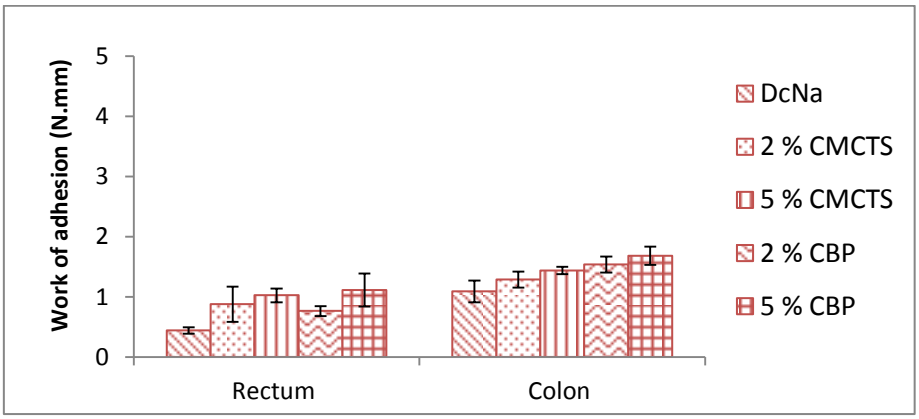


Figure 5.11 : Effect of different segments (rectum and colon) of the porcine large intestines on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 2–5 %w/w of CMCTS or CBP using the tensile setup. Values expressed as mean ± SD, n=5-6.

The correlation coefficient of F_{max} between the rectum and colon was found to be 0.910. This indicated that the colon provided a reasonable representation of the rectum in terms of evaluation of bioadhesion properties of suppositories using the

tensile setup. Due to the low yield of rectum membranes (Table 5.3), the colon mucosa was used in subsequent evaluative studies in Section 5.3.3.

5.3.2.2 Shear measurement

Figure 5.12 showed that F_{max} and W_{ad} increased as contact time was increased until 90 s, followed by a decrease in bioadhesion at 120 s. Shojaei et al. (2000) reported that bioadhesion strength plateaued when contact time was increased beyond 120 s, and attributed it to excessive water sorption. In this study, it is likely that a slippery surface was produced as the disc melts, resulting in a decrease in bioadhesion forces with longer contact times; thus 60 s was selected for subsequent studies.

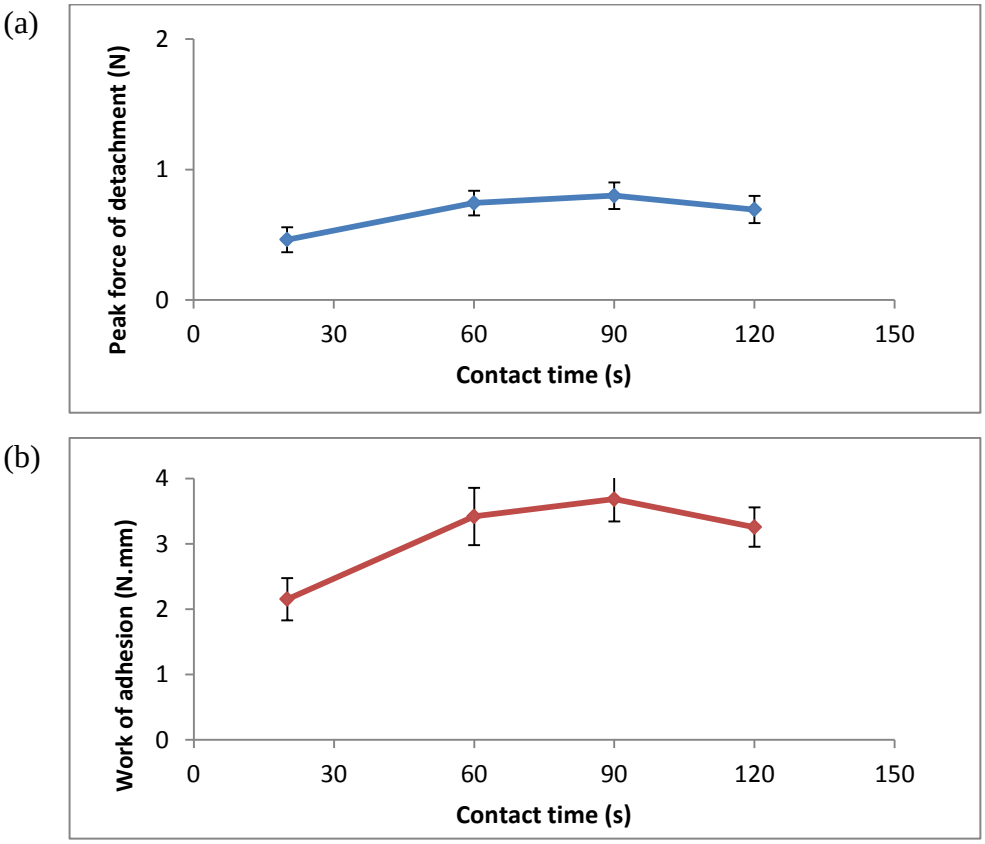
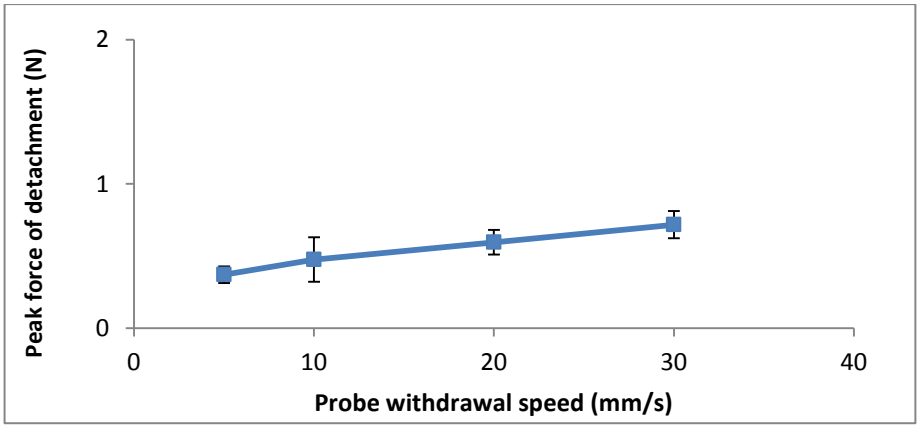


Figure 5.12 : Effect of contact time on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w PVP against the porcine colon mucosa using the shear setup.

Mean ± SD, n=5-6.

The effects of increasing probe withdrawal speed on both the F_{max} and W_{ad} (Figure 5.13) were similar to findings from the tensile setup. Increment in probe withdrawal speed from 5 mm/s to 10, 20 and 30 mm/s resulted in statistically significant increase in F_{max} and W_{ad}. Therefore, probe speed of 30 mm/s was selected for subsequent bioadhesion studies using the shear setup.

(a)



(b)

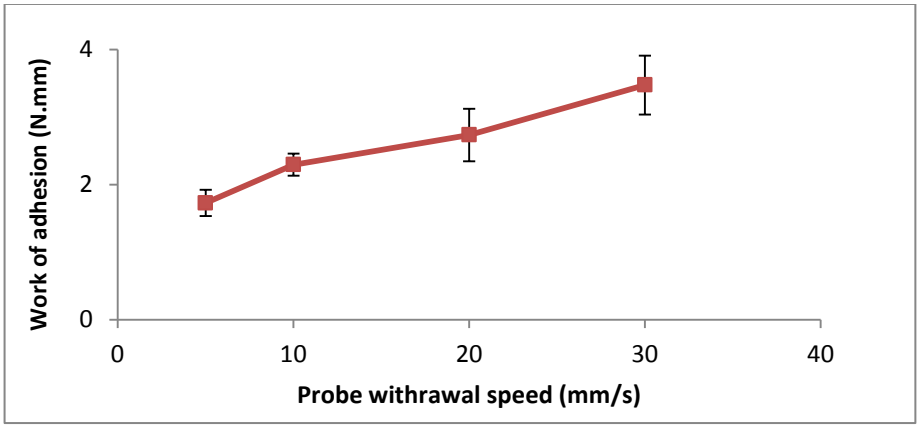
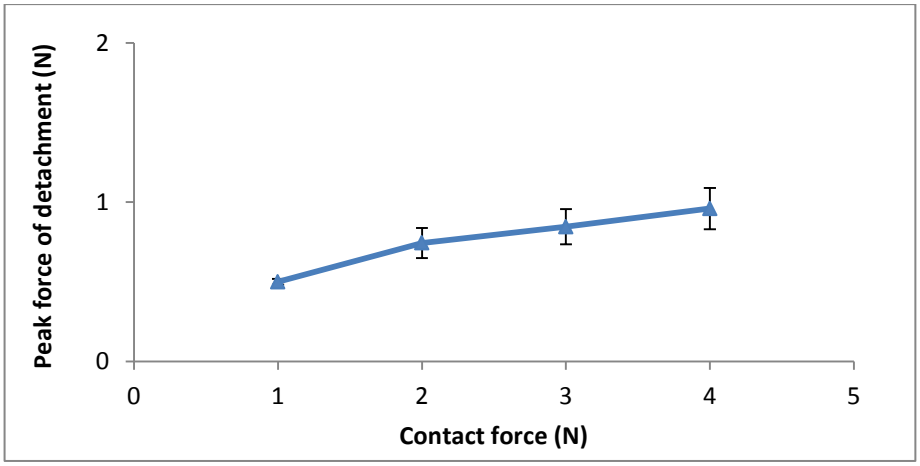


Figure 5.13 : Effect of probe withdrawal speed on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w PVP against porcine colon mucosa using the shear setup. Mean ± SD, n=5-6.

A significant increase in F_{max} and W_{ad} was observed when contact force was increase from 1 to 2, 3 and 4 N (Figure 5.14). There was a ceiling effect for the increment in F_{max} and W_{ad} brought about by increasing contact force, as no significant difference in F_{max} and W_{ad} between contact force of 2, 3 and 4 N. This was in agreement with Wong et al. (1999) where the authors suggested that excessive contact force may lead to mucosal damage without any improvements on bioadhesion. Contact force of 2 N was used in subsequent studies.

(a)



(b)

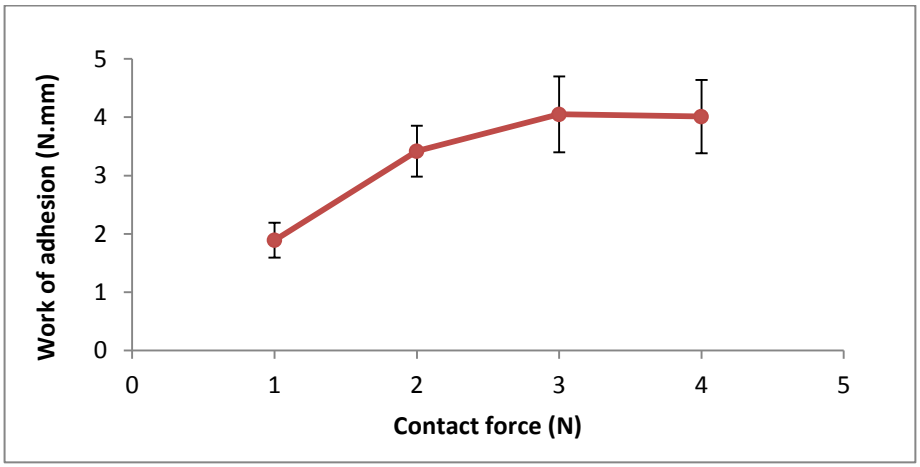
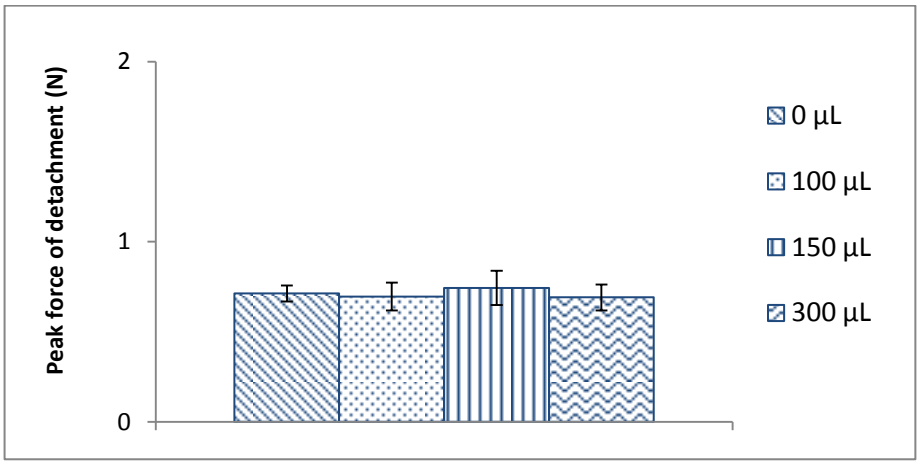


Figure 5.14 : Effect of contact force on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w PVP against the porcine colon mucosa using the shear setup. Mean ± SD, n=5-6.

Figure 5.15 showed that the different volumes of SRM used in this study did not significantly affect the F_{max} and W_{ad} generated. SRM volume of 150 µL was selected for the subsequent studies.

(a)



(b)

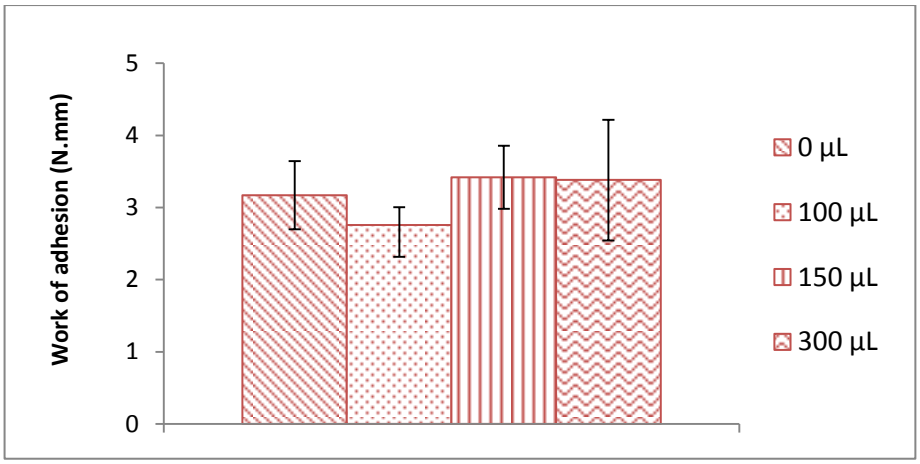
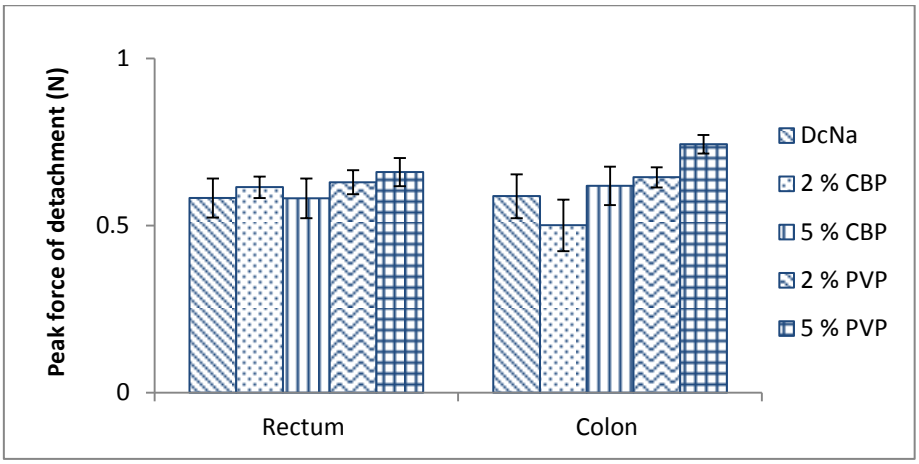


Figure 5.15 : Effect of volume of SRM on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 5 %w/w PVP against the porcine colon mucosa using the shear setup. Mean ± SD, n=5-6.

When different segments of the large intestine (rectum and colon) were investigated, the rank orders of F_{max} and W_{ad} for the tested formulations were similar (Figure 5.16). The correlation coefficient of F_{max} between the rectum and colon using the shear setup was found to be 0.965. As with the observations using the tensile experimental setup

in section 5.3.2.1, the colon mucosa was a suitable replacement of the rectum. Generally, formulations containing either CBP or PVP exhibited higher F_{max} and W_{ad} values than those without polymer, and the bioadhesion conferred by PVP was greater than CBP and increased in a concentration dependant manner.

(a)



(b)

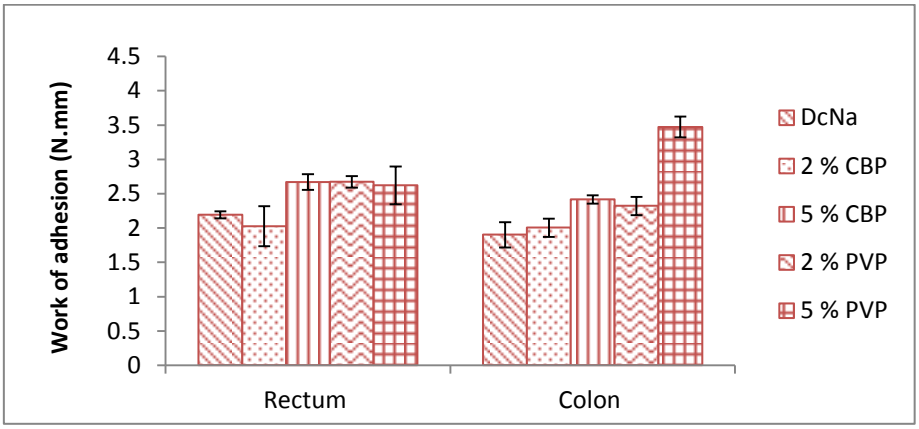


Figure 5.16 : Effect of different segments (rectum and colon) of the porcine large intestines on (a) F_{max} and (b) W_{ad} of SS discs containing 50 mg DcNa and 2–5 %w/w of CBP or PVP using the shear setup. Mean ± SD, n=5-6.

Due to similarity in the data obtained through F_{max} and W_{ad} in both the tensile and shear measurements, only F_{max} was reported in Sections 5.3.3 and 5.3.4.

5.3.3 Evaluation of bioadhesive strength in suppository formulations using biological and synthetic membranes

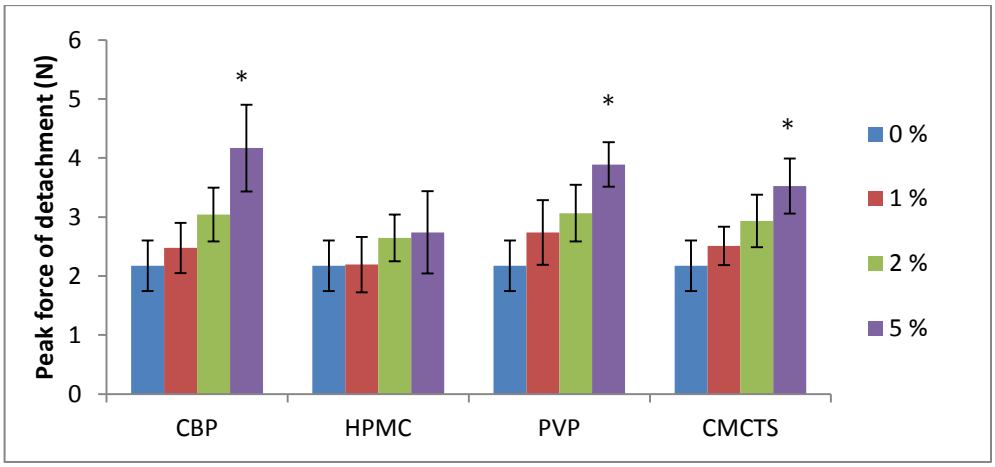
5.3.3.1 Tensile measurement

A contact time of 20 s under a contact force of 2 N, followed by a probe withdrawal speed of 10 mm/s was used in this segment of studies. Porcine colon mucosa spread with 150 μL of SRM was used as a model mucosa. The F_{max} of various bioadhesive suppository formulations are shown in Figure 5.17.

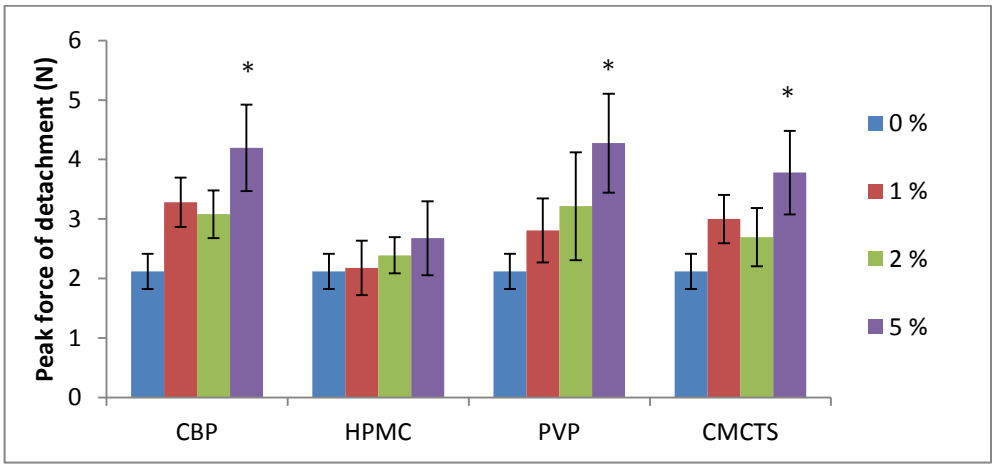
For CB and CE suppositories, only 5 %w/w CBP, 5 %w/w CMCTS and 5 %w/w PVP showed significantly higher F_{max} compared to blank formulations. Although there were small increases in F_{max} for formulations containing 1–2 %w/w of bioadhesive polymers, none of these were statistically significant.

Meanwhile, SS suppositories containing 2–5 %w/w of CBP and PVP and 5 %w/w of CMCTS resulted in a significantly higher F_{max} compared to suppositories without polymers. Formulations with higher amounts of polymer generally exhibited greater bioadhesive properties. The strength of bioadhesion conferred by the polymers was in the ascending rank order of: HPMC < CMCTS < CBP < PVP. Formulations containing HPMC at concentrations of up to 5 %w/w exhibited the weakest bioadhesion (lowest F_{max}) compared to other polymers. Outcomes of the statistical analysis are included in the Appendices 28-30.

(a)



(b)



(c)

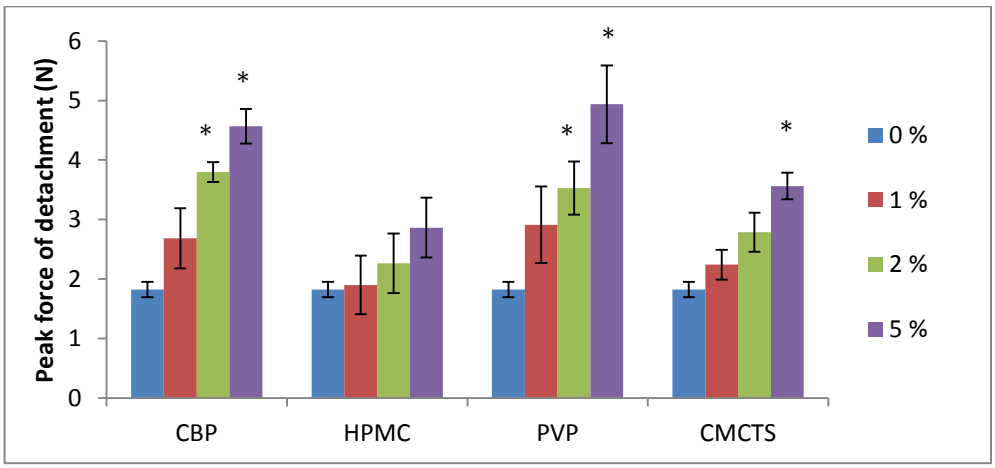


Figure 5.17 : The F_{max} of (a) CB; (b) CE and (c) SS formulations containing 50 mg DcNa and 1–5 %w/w of bioadhesive polymer (CBP, HPMC, PVP, CMCTS) using tensile setup. Asterisks indicate F_{max} values which are significantly different from formulations without bioadhesive polymers. Mean ± SD, n=5–6.

5.3.3.2 Shear measurement

A contact time of 60 s under a contact force of 2 N, followed by a probe withdrawal speed of 30 mm/s was used in this segment of studies. Porcine colon mucosa spread with 150 μL of SRM was used as a model mucosa.

Figure 5.18 showed the F_{max} and W_{ad} measured using the shear setup. In general, bioadhesion measured using the shear setup was observed to increase in the following order: CBP = HPMC < CMCTS < PVP; with formulations containing PVP exhibiting the highest bioadhesive properties. Although all formulations containing 5 %w/w of polymer had significantly higher F_{max}, HPMC exhibited limited bioadhesive properties. This was observed in both the tensile and shear measurements and strongly suggests the limited benefit of using HPMC in the formulation of bioadhesive suppositories. Outcomes of the statistical analysis are included in the Appendices 31-33.

Similar to the tensile setup, formulations containing 5 %w/w PVP was found to generate the highest F_{max}, which indicated the superior bioadhesivity conferred by this particular polymer when incorporated in lipophilic suppositories. The shear forces required to detach sample discs increased with increasing amounts of PVP incorporated into the sample disc. A similar albeit less obvious trend was observed in formulations containing other polymers.

Formulations containing CBP exhibited poor bioadhesive properties when tested using the shear setup; contrary to results obtained using the tensile setup in Section 5.3.3.1.

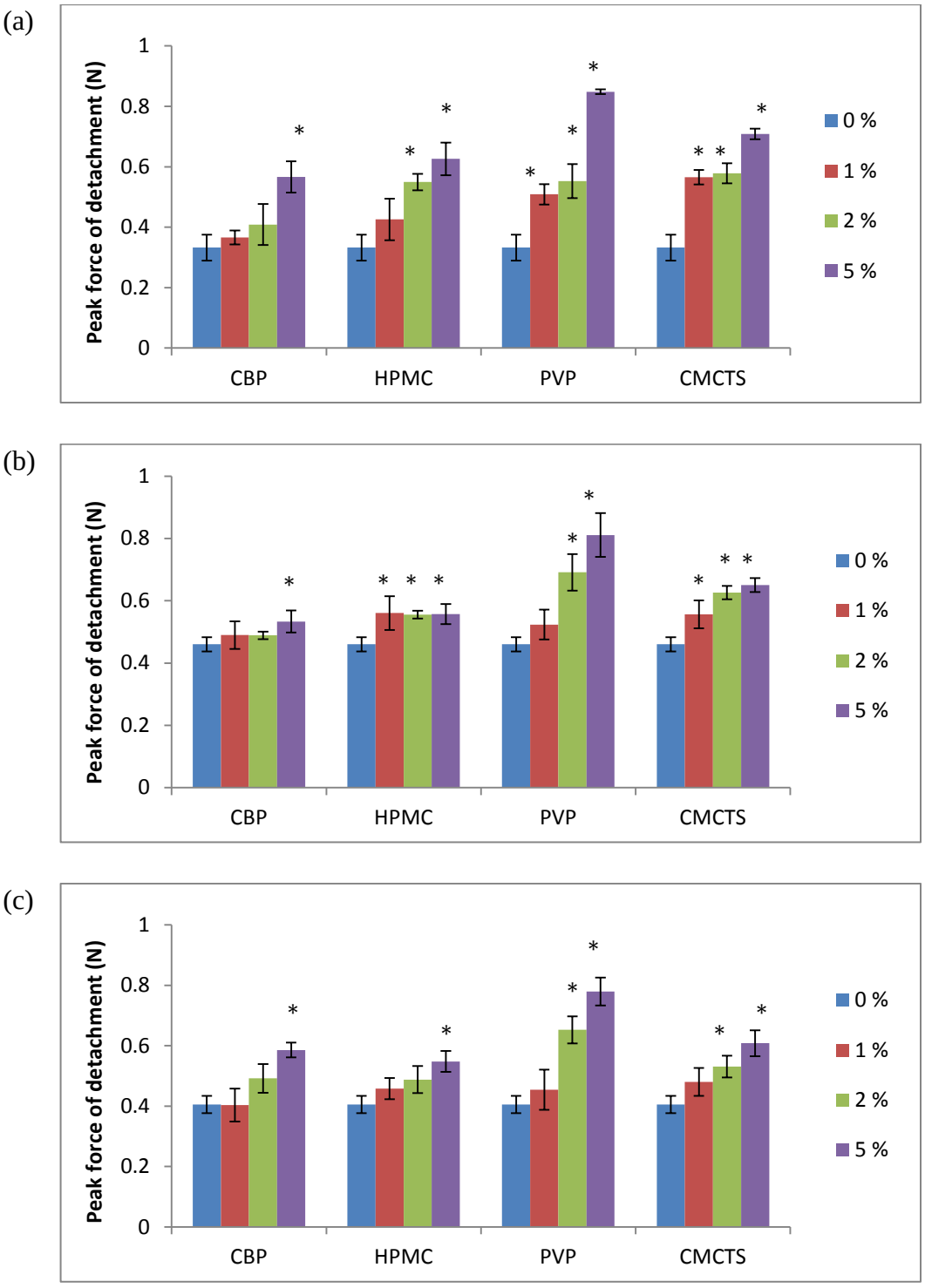


Figure 5.18 : The F_{max} of (a) CB; (b) CE and (c) SS formulations containing 50 mg DcNa and 1–5 %w/w of bioadhesive polymer (CBP, HPMC, PVP, CMCTS) using shear setup. Asterisks indicate F_{max} values which are significantly different from formulations without bioadhesive polymers. Mean ± SD, n=5-6.

The bioadhesion properties of CBP, an anionic polymer is attributed to presence of carboxylic acid (-COOH) groups which facilitates the formation of hydrogen bonds. Lehr and Bouwstra (1992) found that bioadhesion of anionic polycarbophil decreased as the pH of the test medium increased. Furthermore, CBP is known to gel at higher pH. Upon mixing with molten suppository base and simulated rectal fluid (pH 7.4), CBP could have resulted in slippery mucilage which facilitates sliding between the sample disc and mucosa surface; resulting in poor shear F_{max}. Apart from that, this observation could also be a result of the smooth and fine texture of CBP compared to the grainier PVP, CMCTS and HPMC.

Previous studies had inconsistent findings on bioadhesive properties of PVP; Wong et al. (1999) found that PVP K30 produced F_{max} comparable to that of CBP 974P. Conversely, Ivarsson and Wahlgren (2012) reported that PVP had limited bioadhesivity via ellipsometry, tensile strength and rheology methods while Smart et al. (1984) reported poor bioadhesivity in PVP using the Wilhelmy plate method. Current study found that PVP exhibited similar bioadhesive performance compared to CBP in tensile stress measurements and displayed superior bioadhesivity compared to CBP in the shear measurements. PVP, although lack hydrophilic groups, possess cyclic amide groups which could serve as potential sites for hydrogen bonding.

The secondary amine of CMCTS (structure depicted in Figure 1.2c) is protonated to form a positive charge at lower pH while the carboxylic acid groups ionise to form carboxylate groups as the pH increased. At the pH of SRM (pH 7.4), both the amine and carboxylic acid groups would be protonated and could result in formation of temporary bonds between the polymer chains, reducing polymer–mucin interaction

(Hombach and Bernkop-Schnurch, 2010). However, this work found that CMCTS has more promising bioadhesivity compared to CBP when both the tensile and shear measurements were considered collectively.

The poorest bioadhesion was observed in the formulations with HPMC, a linear, nonionic polymer derived from etherified anhydro-glucose rings substituted with a 28–30 % hydrophobic methyl groups. Most of the previous studies which reported of good bioadhesion properties in HPMC formulations employed the lesser methyl substituted HPMC grade 2208 rather than 2910 used in this study (Akbari et al., 2010; Mortazavi and Smart, 1995; Wong et al., 1999). This further affirms the importance of potential hydrogen bonding groups in conferring bioadhesive properties.

5.3.4 Evaluation of synthetic regenerated cellulose membrane as an alternative to biological membrane

Due to variability in mucosa surface properties and difficulties in obtaining biological samples, a synthetic regenerated cellulose membrane was investigated as potential substitute for biological mucosa used during *in vitro* measurement of bioadhesion.

5.3.4.1 Tensile measurement

Figure 5.19 showed that F_{max} generated for the same formulations using synthetic (regenerated cellulose) membrane were much higher than those generated using biological (colon) membranes. However, both were similar in terms of rank order of bioadhesion. The bioadhesivity in ascending manner was found to be: HPMC < CMCTS < CBP < PVP. Formulations tested using the synthetic membranes resulted in smaller SD but were less sensitive compared to the biological membrane, as

observed by the higher F_{max} produced by blank samples. An outcome of the statistical analysis is included in the Appendix 34.

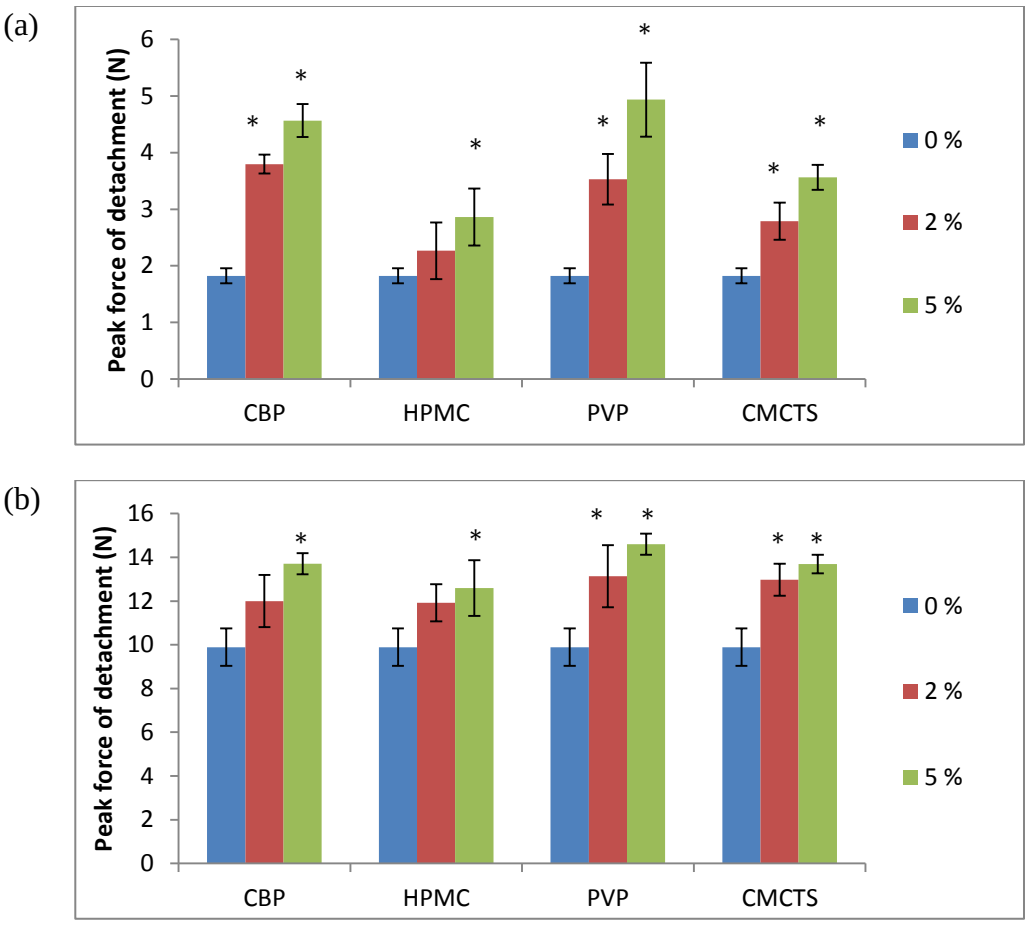


Figure 5.19 : The F_{max} of the SS formulations containing 50 mg DcNa and 0-5 %w/w of bioadhesive polymer (CBP, HPMC, PVP, CMCTS) tested with (a) colon mucosa as biological membrane and (b) synthetic membrane using the tensile experimental setup. Mean ± SD, n=5-6.

The correlation coefficient of F_{max} between the colon and synthetic regenerated cellulose membrane was found to be 0.737. Despite a lower correlation coefficient value, it still indicated a strong relationship between the F_{max} generated by the colon and the F_{max} generated by regenerated cellulose membrane. Despite this strong

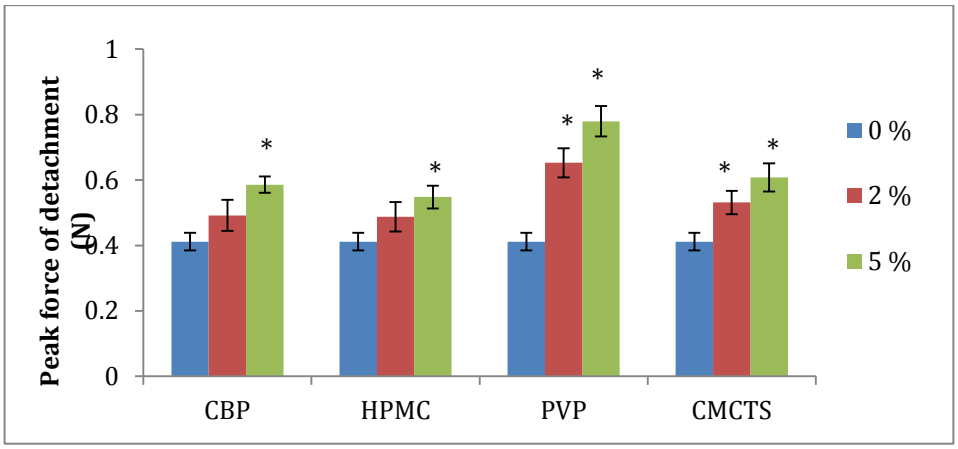
correlation, the usage of synthetic regenerated cellulose as an alternative membrane to colon samples may only be feasible for qualitative comparison (rank order of bioadhesion), as the F_{max} values obtained using synthetic membranes were approximately 3-4 times higher than those obtained using the rectum and colon membranes.

5.3.4.2 Shear measurement

Contrary to the findings from tensile measurements (Section 5.3.4.1); both F_{max} generated using biological and synthetic membranes were comparable and of the same rank order (Figure 5.20). An outcome of the statistical analysis is included in Appendix 35.

The correlation coefficient of F_{max} between the colon and synthetic regenerated cellulose was found to be 0.959. Synthetic regenerated cellulose appeared to be a suitable alternative to biological membrane in *in vitro* bioadhesion studies using the shear setup as there were no marked differences between the results obtained from colonic mucosa and synthetic regenerated cellulose. However, caution has to be exercised while interpreting results in both situations as synthetic membranes have a flat and even surface which is a stark contrast to biological mucosal surfaces.

(a)



(b)

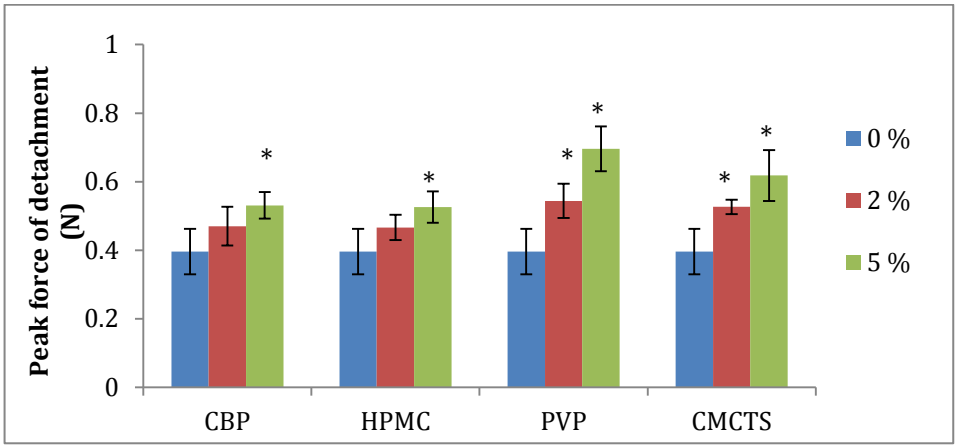


Figure 5.20 : The F_{max} of the SS formulations containing 50 mg DcNa and 0-5 %w/w of bioadhesive polymer (CBP, HPMC, PVP, CMCTS) tested with (a) colon mucosa as biological membrane and (b) synthetic membrane using the shear experimental setup. Mean ± SD, n=5-6.

5.4 Conclusion

Two distinct methods for *in vitro* evaluation of bioadhesive properties of suppositories using the texture analyser were developed and optimised in this chapter. The first method involved the measurement of tensile forces while the second quantified shear forces required to disrupt the bioadhesive bond. Both methods involved temperature control at 37 °C in the presence of SRM to simulate *in vivo* conditions. Formulations containing PVP exhibited superior bioadhesion compared to

the other polymers when subjected to both tensile and shear forces of detachment. This finding was promising for the development of bioadhesive suppositories. Conversely, HPMC exhibited poor bioadhesive properties in both tests and has limited role in the development of bioadhesive suppositories. In addition to evaluation of the bioadhesivity of the formulations, this study also investigated synthetic regenerated cellulose membrane as a practical alternative to biological membranes as mucosa surface for *in vitro* bioadhesion studies. Synthetic regenerated cellulose membranes were generally found to be a good substitute for colon mucosa for qualitative assessment of bioadhesion strengths in both the tensile and shear measurements.

CHAPTER 6

STABILITY STUDIES

6.1 Introduction

6.1.1 Stability studies in suppositories

Suppository formulations are susceptible to both chemical and physical instability when inappropriately stored (Coben and Lordi, 1980; Tukker et al., 1984). Storage temperature and storage duration are common factors causing ageing which leads to altered stability in suppositories (Hosny et al., 1990; Sah and Saini, 2008; Yoshida et al., 1991; Yoshino et al., 1981).

Stability studies fundamentally involve testing both physical and chemical aspects of a particular formulation to determine its shelf life and preferential storage conditions. Physical analysis comprises visual inspection of the physical appearance, mechanical strength (hardness), melting point and softening time, while analysis of active drug compound as well drug release studies make up the crucial aspects of chemical testing.

6.1.2 Chemical testing

Certain drugs are susceptible to chemical degradation or may interact with suppository base after prolonged exposure to warm temperatures (Tukker et al., 1984; Whitworth et al., 1973). When drug degrades, the formulation may no longer be clinically effective and in some cases, the degraded product may even be toxic.

Yoshida et al. (1991) observed that indomethacin suppositories aged by storage at room temperature for one month resulted in slower drug release compared to those stored refrigerated for the same duration of time. A separate study also found that aminophylline suppositories made with CB kept at 30 ºC for 8 weeks resulted in retarded *in vitro* drug release compared to freshly prepared suppositories (Tukker et

al., 1984). Although the authors attributed the slow and incomplete drug release to degradation of aminophylline to theophylline, they also noted that shape of the suppositories remained intact (not melted) for 30 minutes during drug release studies, which could also imply changes in physical properties of the dosage form leading to difficulty in melting or softening.

6.1.3 Physical testing

Lipophilic suppository base in particular, are at risk of a multitude of physical instabilities as they are made up of a mixture of TAG with various polymorphic forms. Visible signs of physical instability include deformation, separation of incorporated additives and active drug from the base as well as the presence of blooming (Khan and Craig, 2004). Bloom occurs as a dull grey surface haze which may sometimes cause the surface of the suppository to feel grainy or crumbly to touch (Allen et al., 2008).

Oleaginous suppositories also undergo transitions into forms exhibiting higher melting points during storage, and these effects appear to be less pronounced when stored at lower temperatures (Hosny et al., 1990; Liversidge et al., 1982; Webster et al., 1998; Yoshino et al., 1981). When its melting point is elevated beyond 37 ºC, a suppository may not melt completely upon administration into the rectum or result in molten with a higher viscosity at body temperature, both occurrences impede drug release (Tukker et al., 1984). Hardening or prolonged softening time of suppositories leads to incomplete melting upon administration into the rectum and may cause local irritation or trigger the defecatory reflex, resulting in expulsion of suppository (Coben and Lordi, 1980).

The effects of ageing in suppositories were highly variable depending on the type of drug and excipients used to formulate the suppositories (Hosny et al., 1990; Yoshino et al., 1982). Furthermore, effects of DcNa and bioadhesive polymers (CBP, HPMC, PVP and CMCTS) towards ageing of suppositories were unknown.

Among formulations developed and tested in the previous chapters, suppositories (CB, CE and SS) containing bioadhesive polymers PVP and CMCTS appear to be promising candidates for fast release DcNa suppositories with bioadhesive properties. Thus, this chapter aims to assess stability of these suppositories as a function of storage duration and storage condition. The suppositories were evaluated and compared in terms of visual appearance, thermal profile, hardness, softening time and DcNa release to ascertain consequences of ageing and the preferred storage conditions in these formulations.

6.2 Materials and methods

6.2.1 Materials

The materials used to manufacture suppository samples used in this chapter have been previously described in Sections 2.1 and 3.2. All other chemicals used have been described in Sections 3.2 and 4.2.

6.2.2 Methods

Suppositories were prepared using methods previously described in Section 3.3.1. Prepared suppositories were either stored refrigerated (3.5 ± 1.5 °C; RH of 29 ± 3%) or kept at room temperature (24.5 ± 2.5 °C; RH 58 ± 5%). The samples were analysed

at three time points; freshly prepared, 100 ± 10 days and 200 ± 10 days on storage until analysis.

6.2.2.1 Physical appearance

The samples were inspected in terms of changes in colour, surface texture or presence of bloom compared to freshly manufactured suppositories. Changes to the suppositories in terms of colour, surface glossiness and smoothness (tactile) after storage for 100 and 200 days were evaluated both visually and by touch.

6.2.2.2 Thermal profile

Thermal analyses of the suppositories were conducted using the DSC system mentioned in Section 2.3.1.1.2. The samples were prepared according to methods described and heated from -10 ºC to 60 ºC. Thermograms were analysed to: (a) determine melting point of the formulations (endothermic peak minimum on thermogram); (b) identify presence of new endothermic peaks; and (c) quantify SFC of the formulations (continuous integration of the thermogram).

6.2.2.3 Hardness

Hardness of suppositories was examined using method described in Section 3.3.2.6. Measurements were repeated with 6 independent samples (n=6) for each of the formulation tested.

6.2.2.4 Softening time

The softening time of suppositories was determined using method described in Section 3.3.2.7. Experiment was carried out in triplicates (n=3) for each formulation.

6.2.2.5 DcNa release

The release of DcNa from aged suppository samples was investigated using method described in Section 4.2.1. Drug release studies (n=3) were carried out for 180 minutes.

6.2.2.6 Statistical analysis of data

The results from hardness (Section 6.2.2.3) and softening time (Section 6.2.2.4) were subjected to analysis of variance (ANOVA) followed by post hoc Tukey's HSD test to detect presence of significant differences between the formulations (freshly prepared samples; samples stored refrigerated for 100 and 200 days; and samples stored at room temperature for 100 and 200 days). The results from DcNa release (Section 6.2.2.5) on the other hand, were analysed via visual comparison of the DcNa release curves alongside DE and MDT.

6.3 Results and discussion

6.3.1 Physical appearance

The physical appearance of PVP and CMCTS suppositories made using CB, CE and SS bases were examined (Table 6.1). There was a general trend of decreasing surface glossiness and increasing graininess of the suppositories stored at room temperature over a period of 200 days. This effect was more predominant in suppositories containing 5 %w/w PVP. Suppositories which were kept refrigerated have less detectable physical changes compared to freshly made samples.

Table 6.1: The physical appearance of PVP and CMCTS suppositories containing 50 mg DcNa after storage at various conditions up to 200 days.

Formulations		Freshly prepared		100 days				200 days			
				Refrigerated		Room temp		Refrigerated		Room temp	
		Surface	Texture	Surface	Texture	Surface	Texture	Surface	Texture	Surface	Texture
CB	No polymer	+++	***	+++	***	++	**	+++	***	++	**
	5%w/w PVP	+++	***	+++	***	++	*	+++	***	+	*
	5%w/w CMCTS	+++	***	+++	***	++	**	+++	***	+	**
CE	No polymer	+++	***	+++	***	++	***	+++	***	+	**
	5%w/w PVP	+++	***	+++	***	++	**	+++	***	+	*
	5%w/w CMCTS	+++	***	+++	***	++	***	+++	***	+	**
SS	No polymer	+++	***	+++	***	++	***	+++	***	+	**
	5%w/w PVP	+++	***	+++	***	++	**	+++	***	+	*
	5%w/w CMCTS	+++	***	+++	***	++	***	+++	***	+	**

‘+’ denotes glossiness of the suppository surface, with ‘+++’ glossy and ‘+’ dull; while ‘*’ denotes smoothness of the suppository to touch, with ‘***’ smooth and ‘*’ grainy.

6.3.2 Thermal profile

Due to the natural composition of fats, their polymorphic transitions often involve multiple TAG which by themselves exists in an array of polymorphs. Changes in properties of fats have been mainly attributed to polymorphic transitions and segregation of components within complex lipids.

Figures 6.1(ii-iii) showed that the CB suppositories containing DcNa and 5 %w/w PVP which were kept refrigerated (3.5 ± 1.5 ºC) for up to 200 days did not result in a change in melting point (endothermic peak). However, the onset of melting for these suppositories shifted to a lower temperature, as demonstrated by the widening of the endothermic peak (Figure 6.1, arrow). A similar observation was demonstrated in CB suppositories containing DcNa and 5 %w/w CMCTS in Figure 6.2. This a potential cause of concern as excessively low onset of melting could result in suppositories liquefying during handling prior to insertion into the rectum.

On the other hand, melting point of CB suppositories kept at room temperature increased throughout storage (Figures 6.1(iv-v) and Figures 6.2(iv-v)). This increase in melting point was observed as early as 100 days of storage at room temperature. The melting point was 34.5 and 35.1 ºC for suppositories stored at room temperature for 100 and 200 days respectively; compared to freshly prepared suppositories which melted at 32.9 ºC. This was believed to be due to gradual transformation of polymorphic forms 3B and 4A to the stable 4B (nomenclature as described in Table 2.1), which was a commonly observed polymorphic transition in poorly stored chocolates (Lonchampt and Hartel, 2004). Increment in melting point of suppositories stored at room temperature was reflected as a rightward shift in the SFC curve in Figure 6.3.

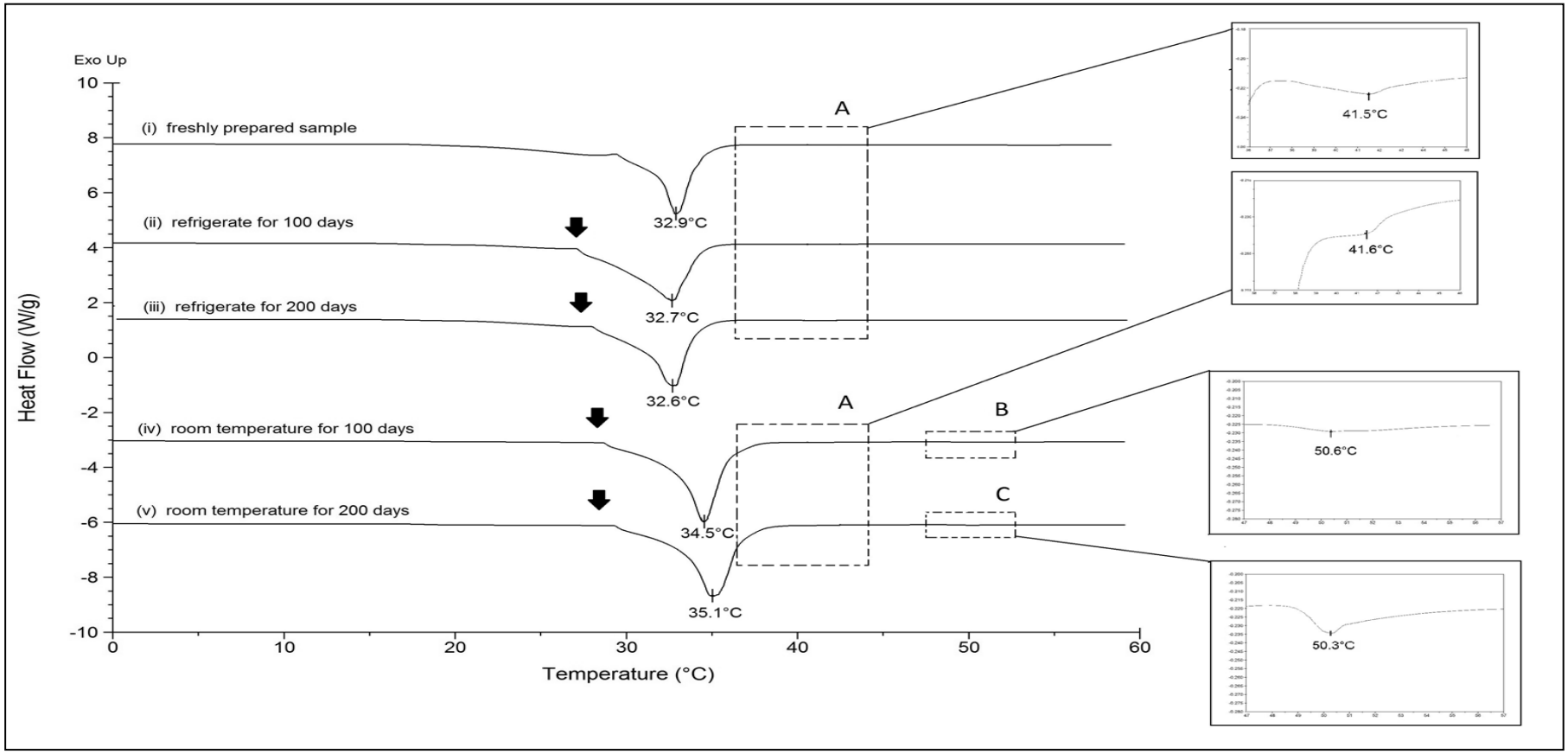


Figure 6.1 : The DSC thermogram of CB suppositories containing 50 mg DcNa and 5 %w/w PVP. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated at for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

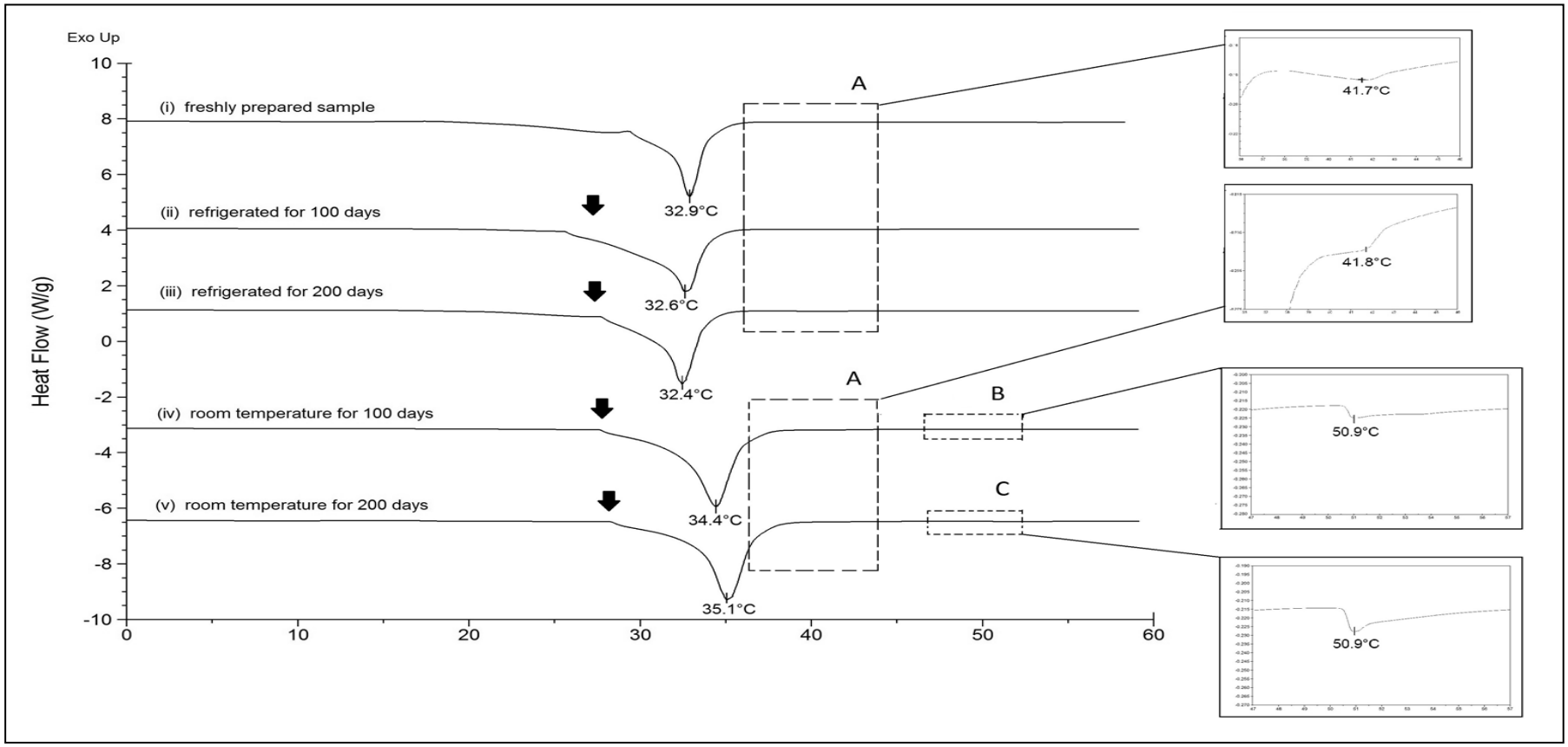
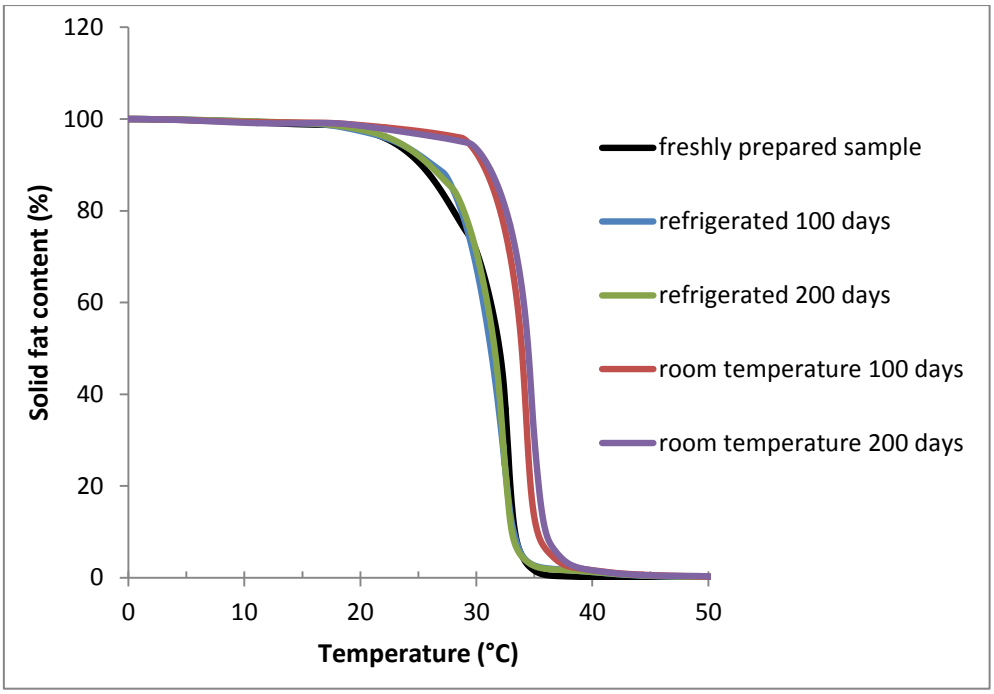


Figure 6.2 : The DSC thermogram of CB suppositories containing 50 mg DcNa and 5 %w/w CMCTS. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated at for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

(a)



(b)

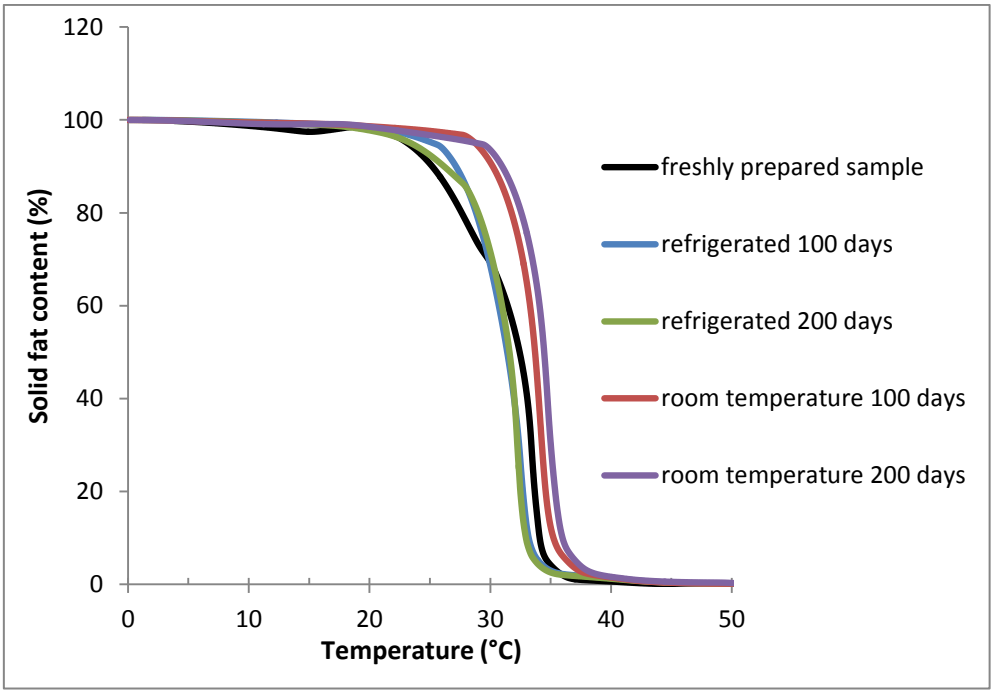


Figure 6.3 : The SFC of CB suppositories containing 50 mg DcNa and (a) 5 %w/w PVP and (b) 5 %w/w CMCTS.

A faint shoulder at approximately 42 ºC (Figures 6.1-6.2, Section A inset) was observed in all freshly prepared, refrigerated and room temperature samples. This is likely to be due to the presence of small amounts of the β form of the stearic acid-oleic acid-stearic acid (SOS) TAG which were already present in the CB stock used to manufacture suppositories. The melting points of CB TAG and their polymorphic forms are tabulated in Table 6.2.

Table 6.2 : The melting points of various polymorphic forms of CB TAG. (Fatty acid nomenclature under TAG column as follows: P = palmitic acid; O = oleic acid; S = stearic acid)

TAG	Composition in CB (%) (Lonchampt and Hartel, 2004)	Melting Points of Polymorphic forms (°C) (Arishima et al., 1991; McClements, 1999; Sato, 2001; Smith, 2009; Susumu and Konishi, 2011)						
		α	γ	δ	β’		β	
					β2’	β1’	β2	β1
POS	46.9	19.5		28.3	31.6		35.5	
SOS	29.8	23.5	35.4		36.5		41.0	43.0
POP	12.6	15.2	27.0	29.2	30.3	33.5	35.1	36.7
POO	11.0	-4.0			18.2 – 19.0			
SOO	1.8	24						

Furthermore, a small endotherm was observed at 50 ºC (Figures 6.1-6.2, Sections B and C; insets iv-v). This endotherm is believed to be due to increased fraction of saturated TAG as a result of subsequent storage of CB suppositories at room temperature. Loisel et al. (1998) observed that lipid segregation occurred in CB during

storage at 30 ºC which resulted in crystallisation of a saturated TAG, in addition to the usual form V polymorph (Forms 4A and 4B as per nomenclature in Table 2.1). The melting point of polymorphic forms observed in various saturated TAG was tabulated in Table 6.3.

Table 6.3: The melting points of various polymorphic forms of saturated TAG.

TAG	**Melting point of polymorphic forms (ºC)** (Belitz et al., 2009; Da Silva et al., 2009; Sato and Kuroda, 1987)		
	α	**β'**	**β**
Trilaurin	15.2	34	46.5
Trimyristin	32.8	45	58.5
Tripalmitin	44.7	56.5	66.4
Tristearin	54	65	72.5

Figures 6.4-6.7 showed the thermal profile of 5 %w/w PVP and CMCTS suppositories made using HPKS (CE and SS). The progression of thermal changes in both CE and SS were similar and findings from Figures 6.4-6.7 will be discussed using CE suppositories containing DcNa and 5 %w/w PVP as reference (Figure 6.4).

CE suppositories containing 5 %w/w of PVP (Figure 6.4) which were kept refrigerated for 100 and 200 days (melting point= 34.0 ºC on both occasions) did not show any significant changes in melting point compared to freshly prepared samples (melting point=33.9 ºC). The melting points were similar and there were no additional endothermic or exothermic events even up to 200 days of refrigeration (3.5 ± 1.5 ºC; RH 29 ± 3 %).

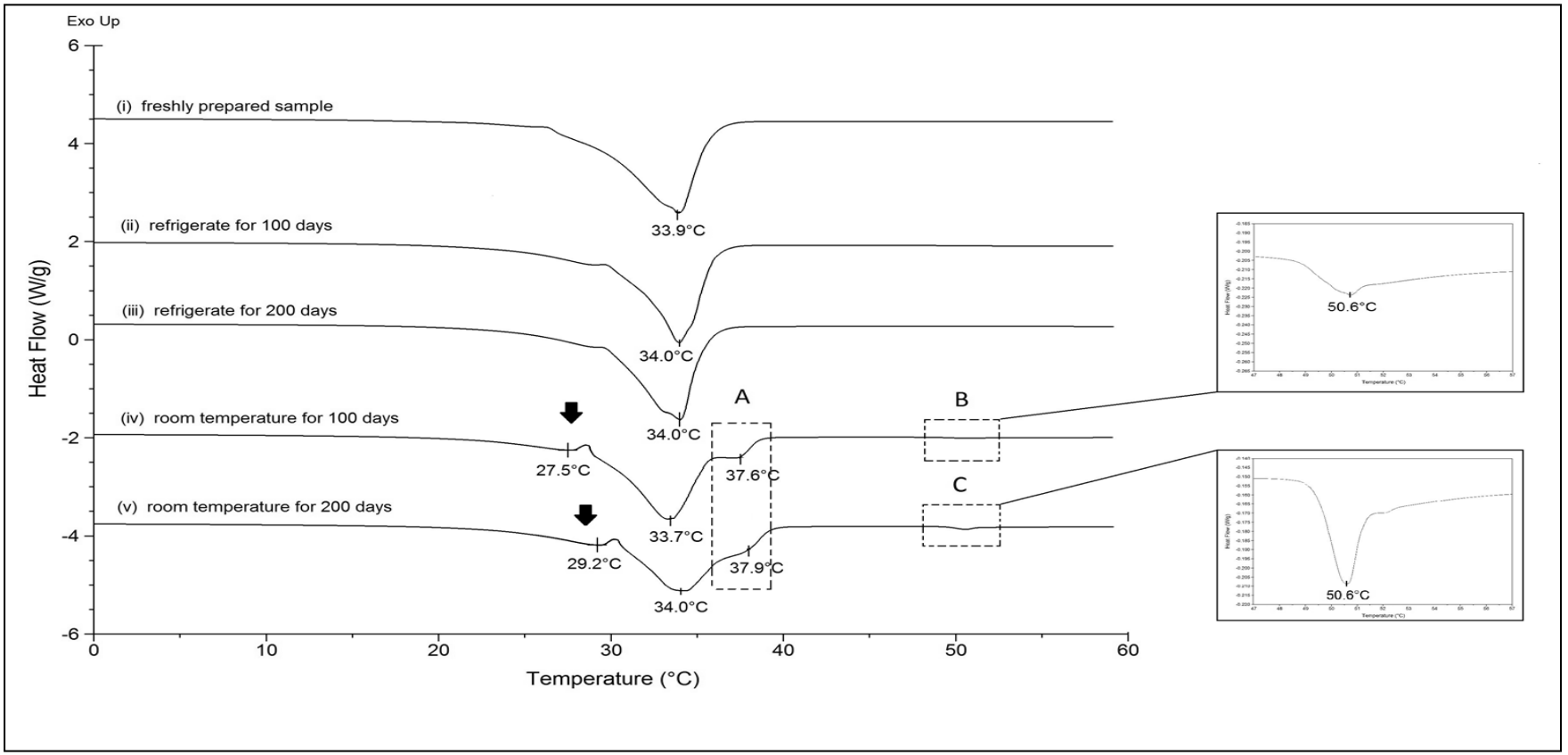


Figure 6.4 : The DSC thermogram of suppositories made using CE as suppository base containing 5 %w/w PVP. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated at for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

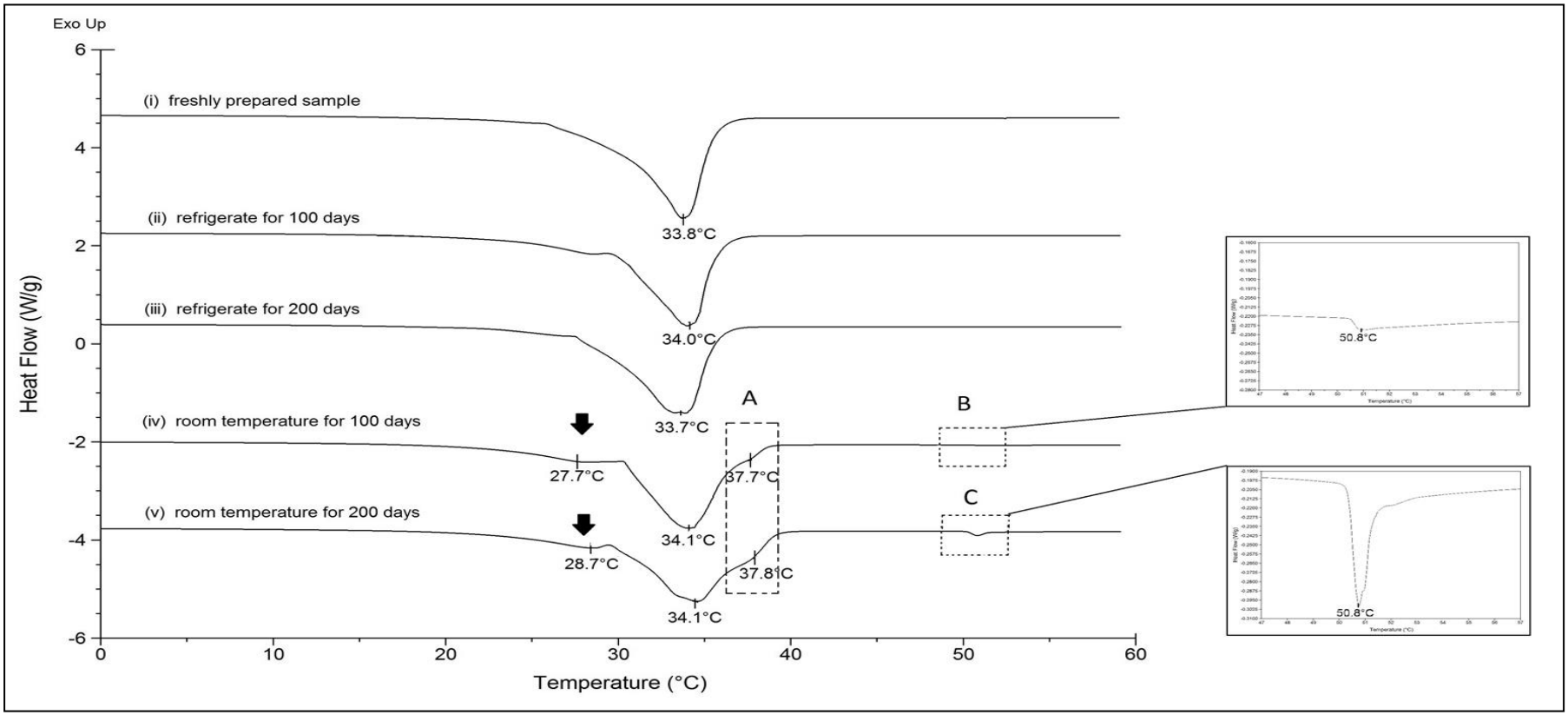


Figure 6.5 : The DSC thermogram of CE suppositories containing 50 mg DcNa and 5 %w/w CMCTS. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated at for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

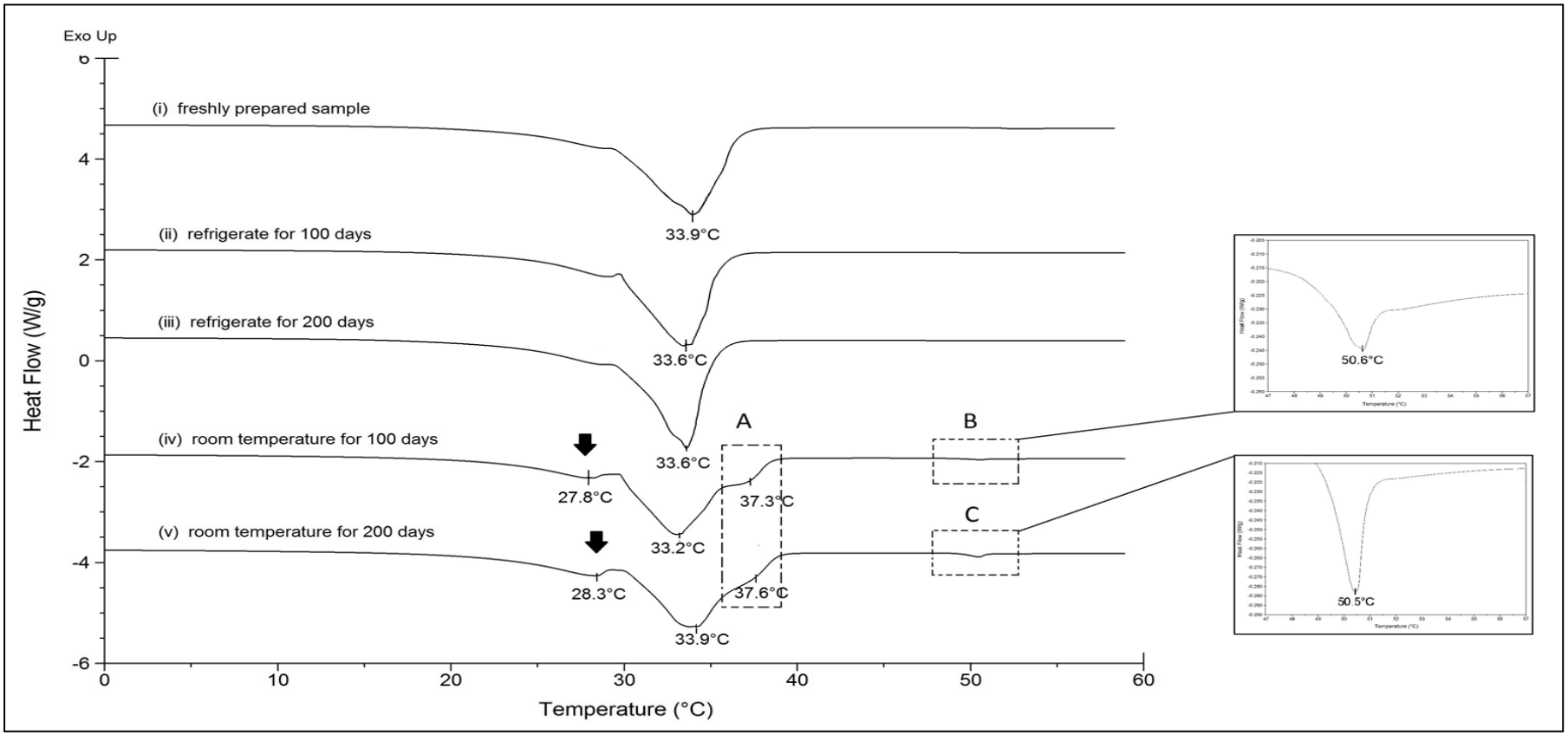


Figure 6.6 : The DSC thermogram of SS suppositories containing 50 mg DcNa and 5 %w/w PVP. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

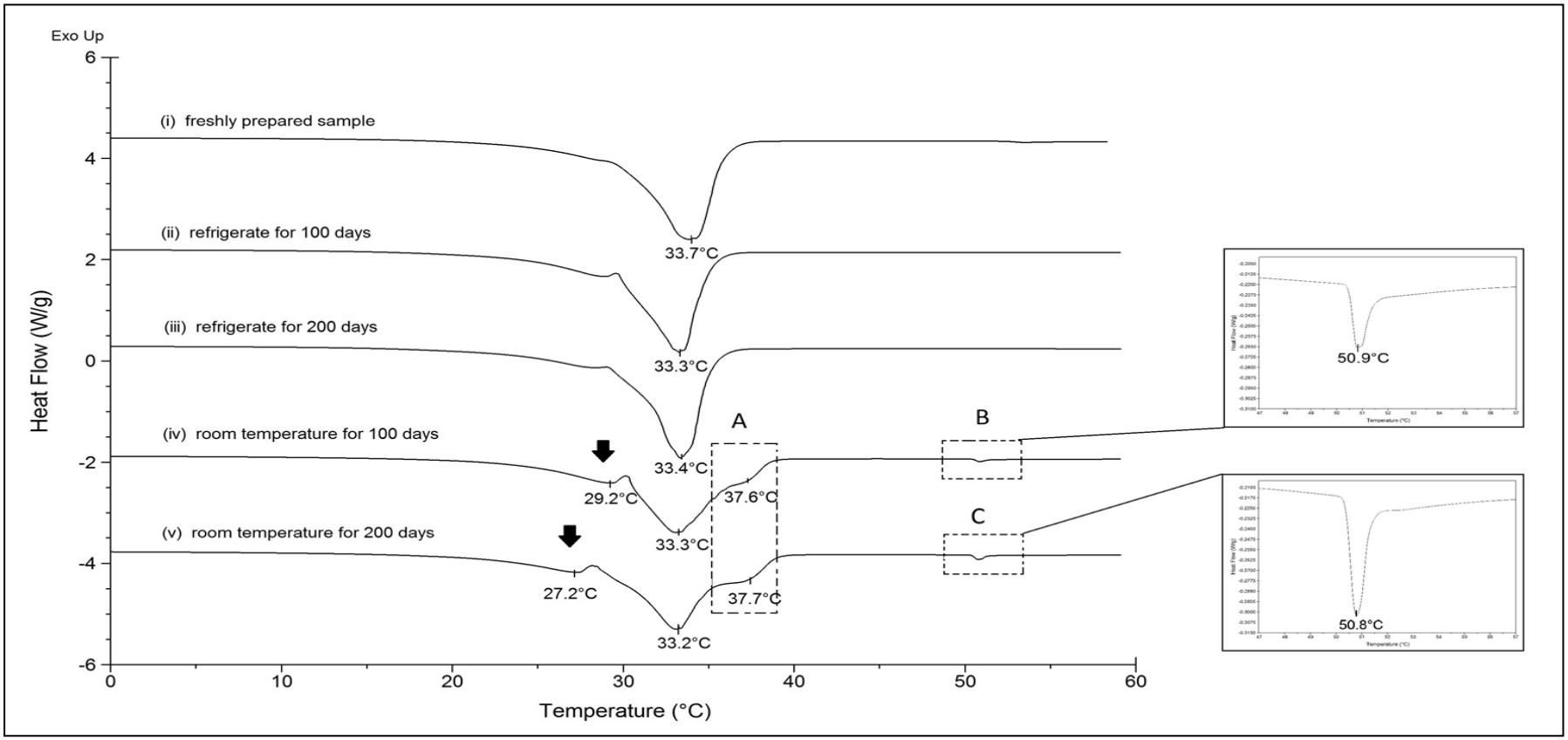


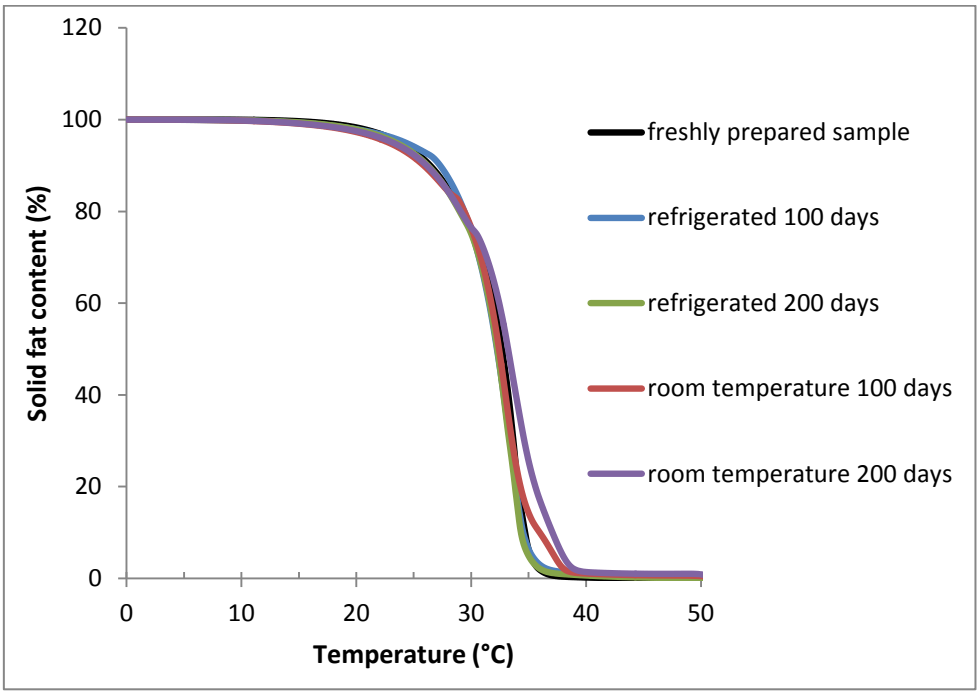
Figure 6.7 : The DSC thermogram of SS suppositories containing 50 mg DcNa and 5 %w/w CMCTS. Individual thermograms show the melting endotherm of suppositories which were (i) freshly prepared; stored refrigerated for (ii) 100 days and (iii) 200 days; stored at room temperature for (iv) 100 days and (v) 200 days. Inset shows enlarged portions of the thermogram.

Conversely, an additional peak was observed at 27.3 and 29.4 ºC in suppositories stored at room temperature for 100 and 200 days respectively (Figures 6.4(iv-v), arrow). These peaks were likely due to separation of lower melting point TAG from the complex multicomponent mixture which makes up HPKS. Furthermore, another shoulder peak was observed at approximately 38 ºC in suppositories kept at room temperature (Figures 6.4(iv-v); Section A). This could be a result of gradual β stabilisation of trilaurin (C_{36}) which is present as 26–29 % of HPKS content (Chin et al., 2003; Smith et al., 2004; Siew, 2001).

An endothermic event was observed at 50 ºC (Figure 6.4, insets B and C) but at a bigger magnitude compared to CB. This is probably due to the melting of trisaturated TAG. CB contains approximately 3 % of trisaturated TAG while HPKS contains approximately 7 % these high melting trisaturated TAG (Smith et al., 2004). The amount of TAG component which melts at 50 ºC in samples stored at room temperature increased from 100 days (Figure 6.4, inset B) to 200 days (Figure 6.4, inset C) of storage. This was consistent in all the HPKS formulations, suggesting that storage at room temperature resulted in an increased formation of this TAG species.

The SFC curves of CE and SS suppositories (Figures 6.8-6.9) stored under refrigeration and at room temperature were similar up to 35 °C; after which the curve for suppositories stored at room temperature shift rightward, indicating the presence of TAG with a higher melting point. This was different from CB suppositories where the entire SFC curve shifted to a higher temperature (Figure 6.3).

(a)



(b)

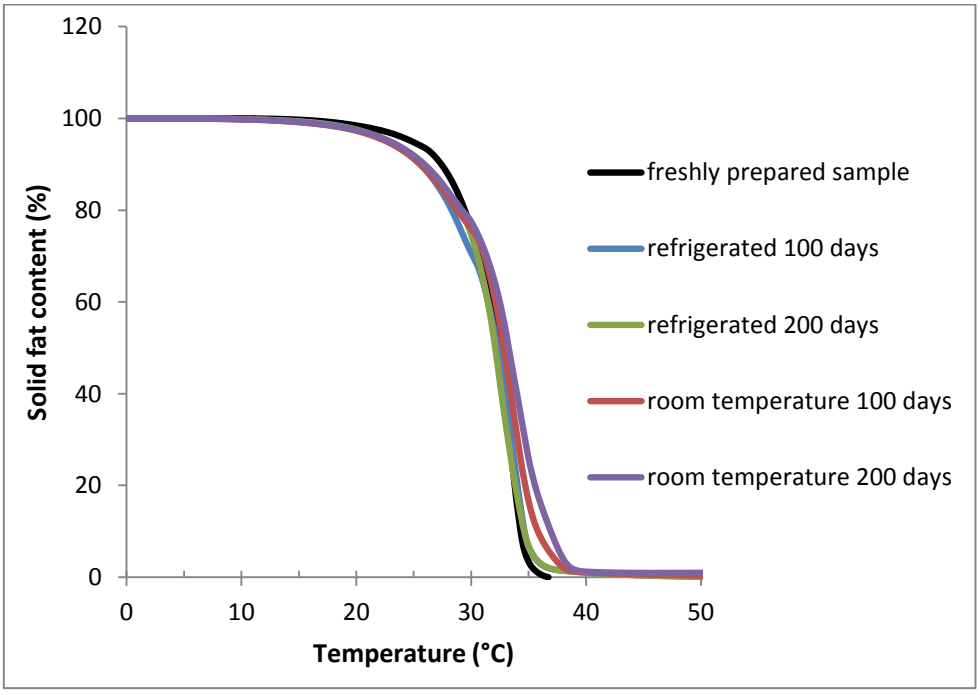
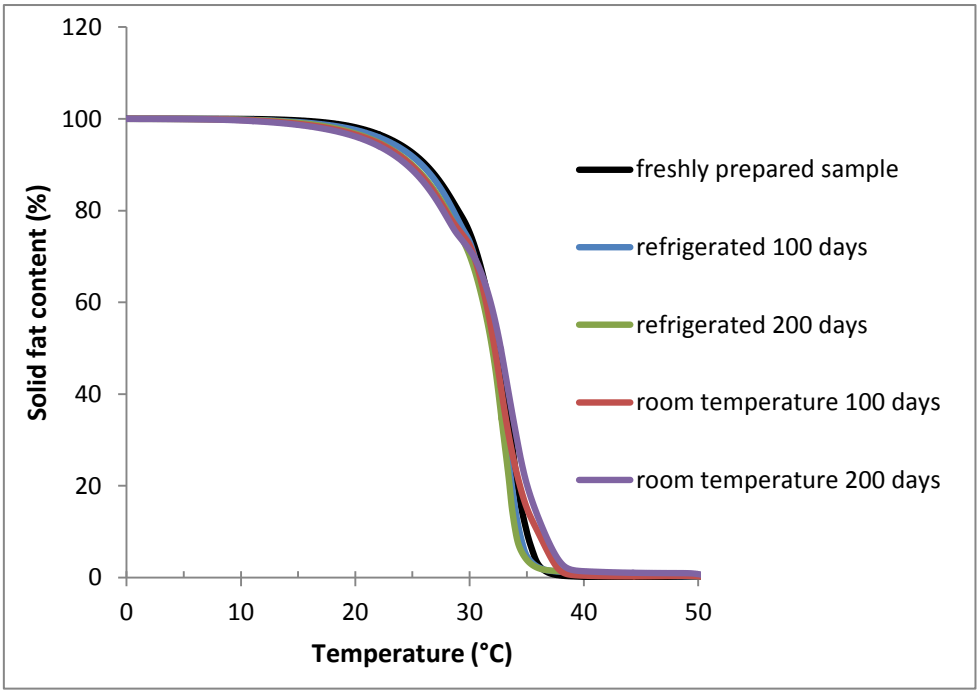


Figure 6.8 : The SFC of CE suppositories containing 50 mg DcNa and (a) 5 %w/w PVP and (b) 5 %w/w CMCTS.

(a)



(b)

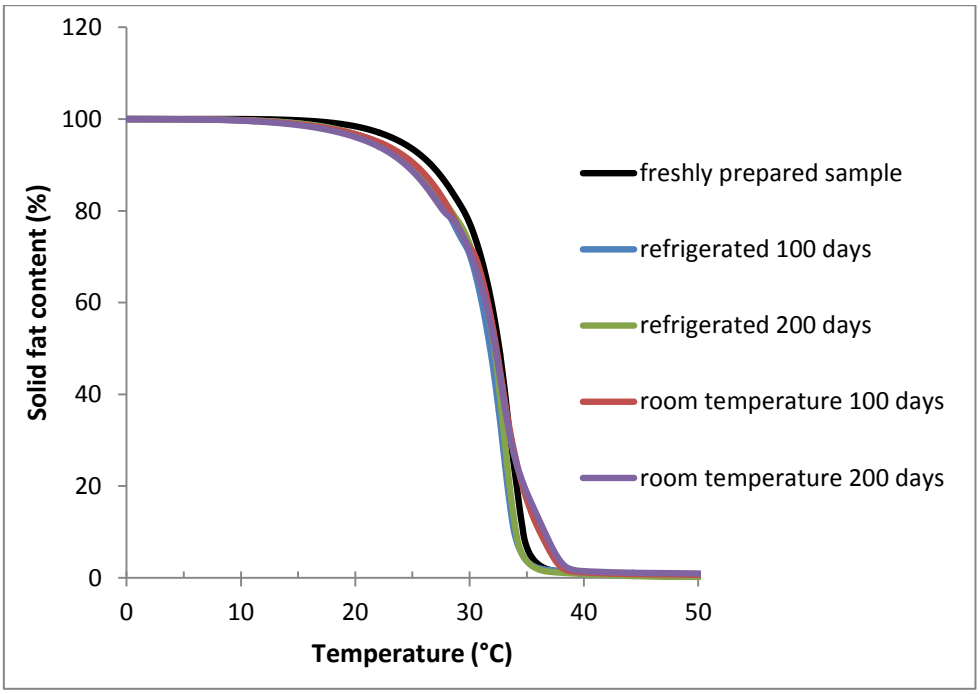


Figure 6.9 : The SFC of SS suppositories containing 50 mg DcNa and (a) 5 %w/w PVP and (b) 5 %w/w CMCTS.

6.3.3 Hardness

Figures 6.10a-c showed changes in hardness of suppositories stored under different storage conditions for different durations.

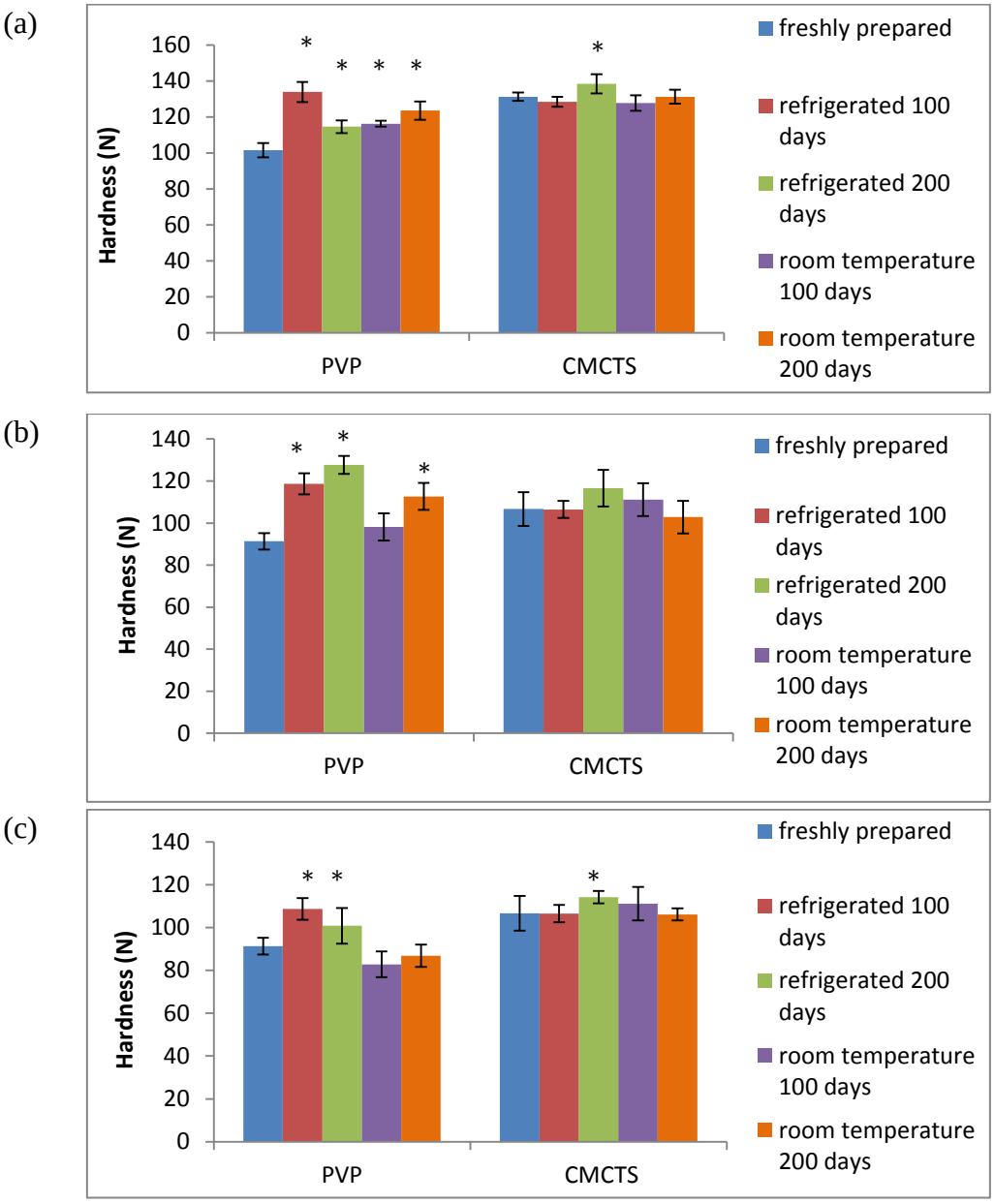


Figure 6.10 : The hardness of suppositories made using (a) CB; (b) CE and (c) SS containing 50 mg DcNa and 5 %w/w PVP and 5 %w/w CMCTS after various storage conditions up to 200 days. Asterisks indicate a significant difference in hardness from 'freshly prepared' suppositories. Mean ± SD, n=6.

Refrigerated suppositories containing 5 %w/w PVP demonstrated statistically significant increase in hardness at both 100 and 200 days ($p < 0.05$). In general, refrigerated 5 %w/w PVP suppositories were harder than suppositories kept at room temperature for the same period.

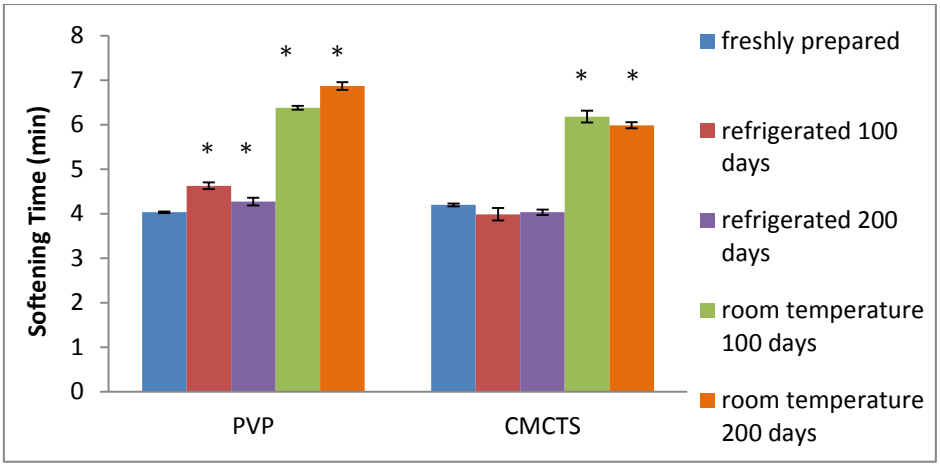
Meanwhile, suppositories with 5 %w/w CMCTS showed very minimal differences in hardness even when stored for 200 days both refrigerated and at room temperature. A significant increase in hardness was only observed in CB and SS suppositories containing 5 %w/w CMCTS after refrigeration for 200 days. Statistical comparison of the formulations using Tukey's HSD analysis is tabulated in Appendix 36.

Sah and Saini (2008) found that lipophilic indomethacin suppositories made using Mayol W45 and Hydrokote AP5 became harder after being subjected to freeze-thaw cycles or accelerated stability test at 30 ºC. The current work however, did not find a clear trend of changes in hardness of the suppositories containing 5 %w/w of PVP and CMCTS against storage time and storage conditions.

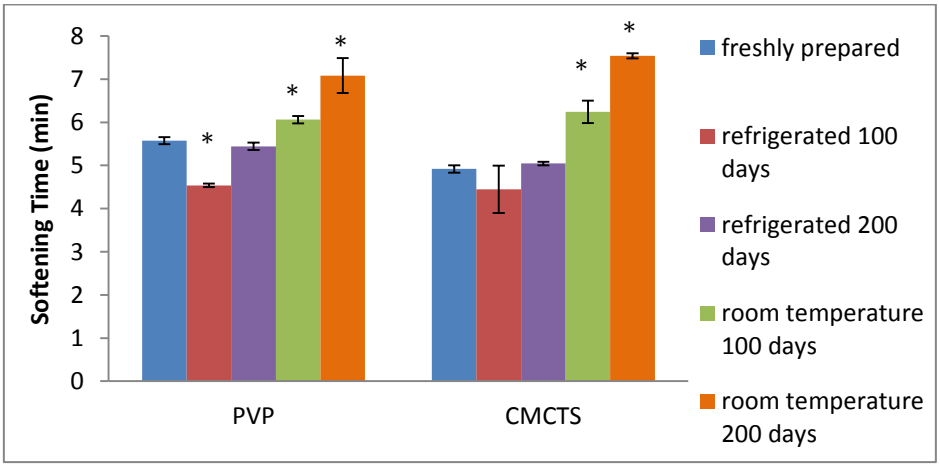
6.3.4 Softening time

Figure 6.11 showed that suppositories stored at room temperature have longer softening times. This was consistent for suppositories containing both PVP and CMCTS. All the formulations stored at room temperature for 100 and 200 days (except SS suppositories containing 5 %w/w PVP at room temperature for 100 days) had significantly prolonged softening times compared to freshly prepared suppositories.

(a)



(b)



(c)

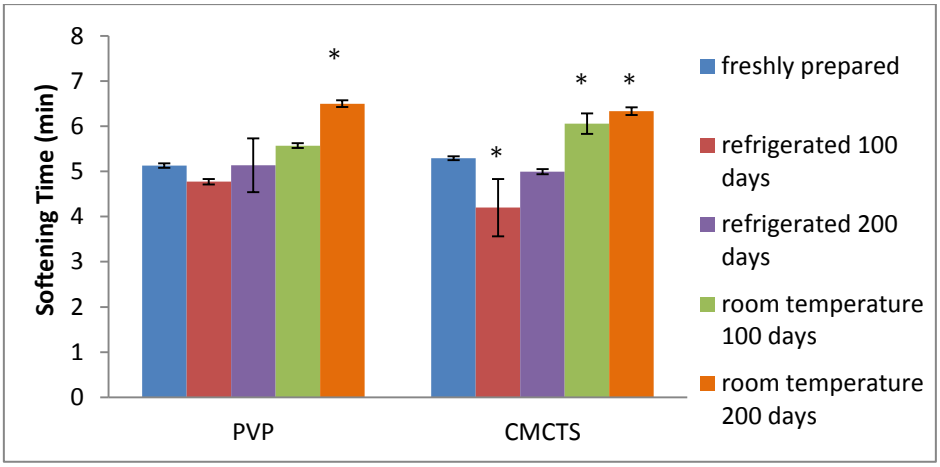


Figure 6.11 : The softening time of suppositories made using (a) CB; (b) CE and (c) SS containing 50 mg DcNa and 5 %w/w PVP and 5 %w/w CMCTS under different storage conditions. Asterisks indicate a significant difference in softening time compared to 'freshly prepared' suppositories. Mean ± SD, n=6.

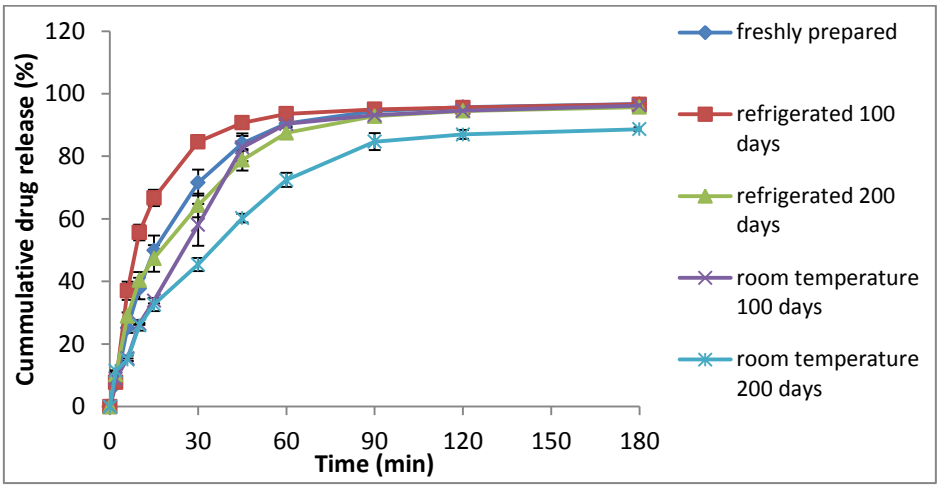
With the exception of CB suppositories containing 5 %w/w PVP (Figure 6.11a), all other refrigerated formulations have shorter softening times compared to freshly prepared samples. This observation however, was only statistically significant in CE suppositories containing 5 %w/w PVP and SS suppositories containing 5 %w/w CMCTS after refrigeration for 100 days. Statistical comparison of the formulations using Tukey's HSD analysis is tabulated in Appendix 37.

Moes and Jaminet (1976) observed marked increase in liquefaction time of suppositories measured at 37 ºC after prolonged storage at 30 ºC. However, the same authors also found that increment in liquefaction time may or may not affect rectal absorption of drugs. Other factors such as change in viscosity and melting point as a result of ageing could have brought about a cumulative synergistic or contradictory effect on rectal absorption.

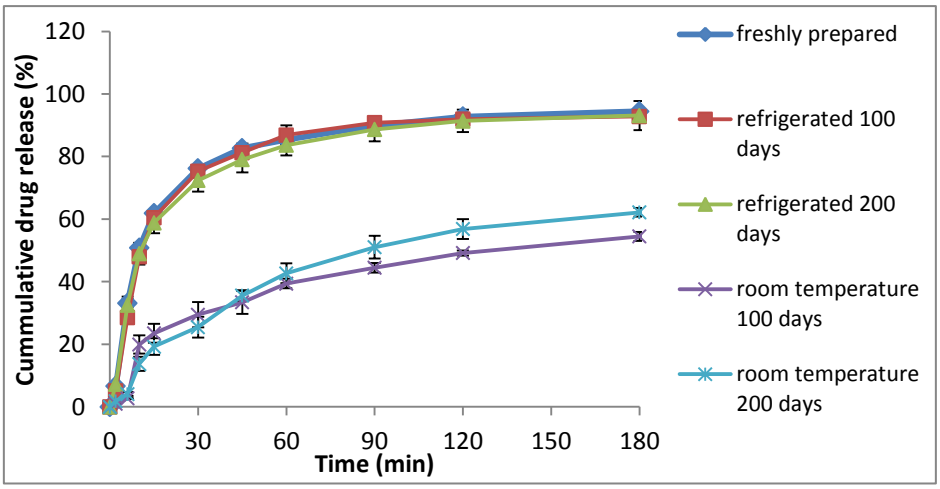
6.3.5 DcNa release

Both Figures 6.12-6.13 showed that refrigeration of suppositories up to 200 days did not alter DcNa release from all the formulations tested, with the exception of CB suppositories containing 5 %w/w CMCTS (Figure 6.13a). Refrigerated CB suppositories containing 5 %w/w CMCTS showed a slight and gradual decrease in rate of DcNa released over time (from freshly prepared to 100 and 200 days). Another study reported reduction in ampicillin release from suppositories stored at 4 ºC over a period of 240 days and the degree of reduction was dependent on type of base used as well as the medicament used (Hosny et al., 1990).

(a)



(b)



(c)

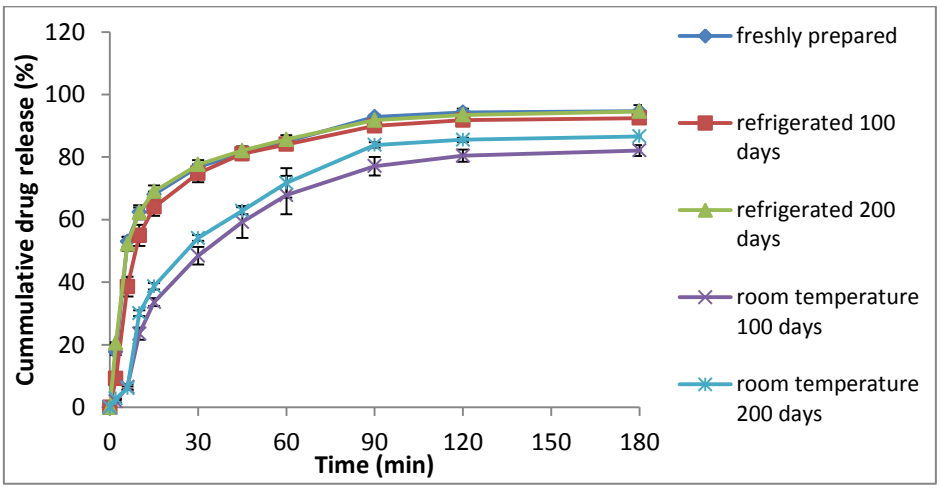


Figure 6.12: The cumulative DcNa release from (a) CB; (b) CE and (c) SS suppositories containing 50 mg DcNa and 5 %w/w PVP under different storage conditions and duration. Mean ± 2 SE, n=3.

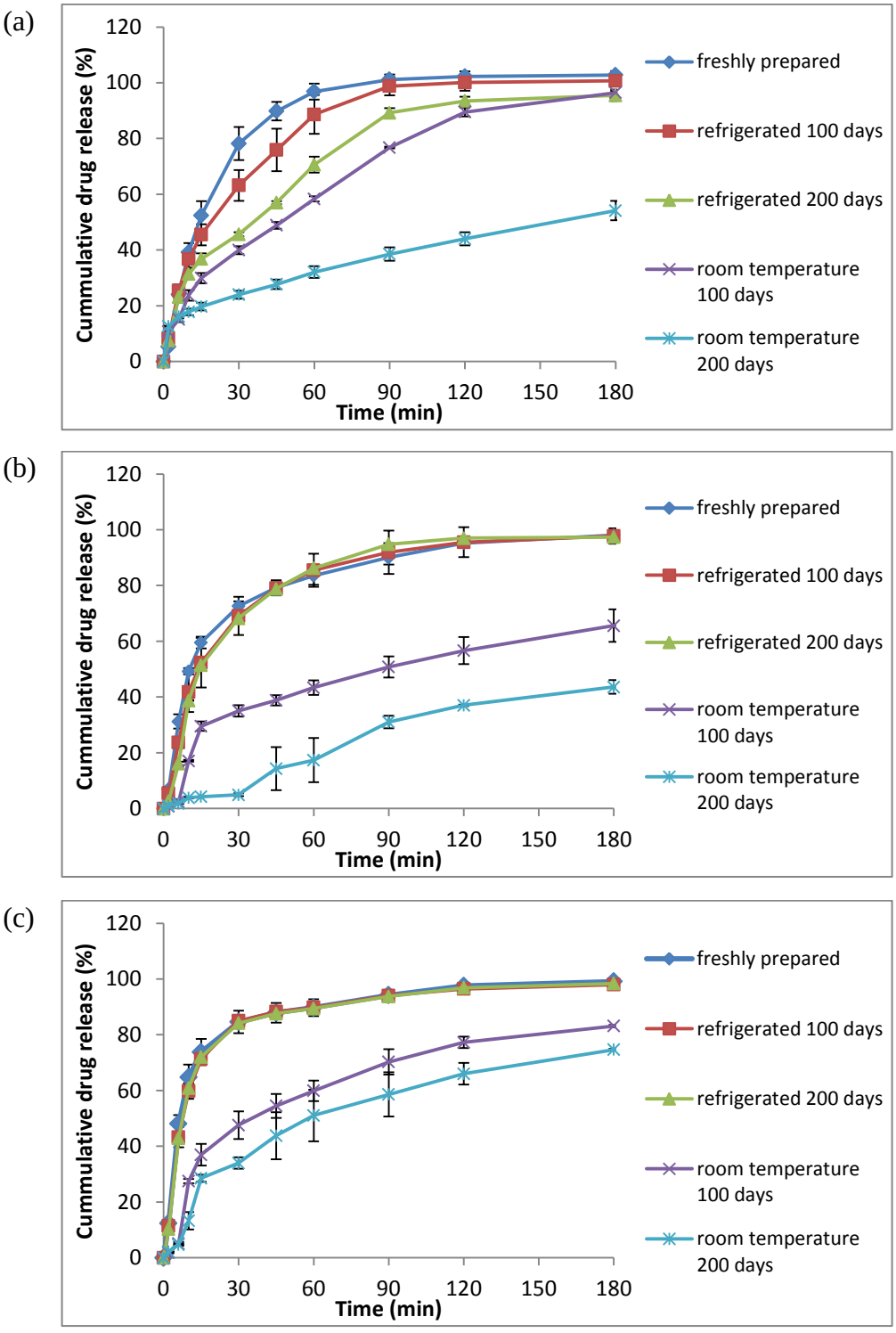


Figure 6.13: The cumulative DcNa release from (a) CB; (b) CE and (c) SS suppositories containing 50 mg DcNa and 5 %w/w CMCTS under different storage conditions and duration. Mean ± 2 SE, n=3.

Conversely, suppositories stored at room temperature for 100 and 200 days generally had lower rates as well as lesser extent of DcNa release at 180 minutes. This observation was independent of type of suppository base (CB, CE and SS) and type of bioadhesive polymer incorporated (PVP, CMCTS). A study by De Blaey and Rutten-Kingma (1977) reported a drastic decrease (50-60 %) in aminophylline release from CB suppositories as early as 4 weeks storage at 22 °C and 6 days at 30 °C.

The parameters of drug release were numerically presented as the DE and MDT values in Tables 6.4-6.5 respectively for easier comparison. Suppositories stored at room temperature had significantly lower DE and higher MDT compared to freshly prepared samples and the refrigerated samples. Among the suppositories stored at room temperature, formulations containing 5 %w/w CMCTS have lower DE and longer MDT compared to those containing 5 %w/w PVP. This suggests that suppositories containing PVP have better resistance towards accelerated ageing conditions although a definite reason for this observation is not known.

The decreased DcNa release could be explained via findings of the DSC thermogram and SFC curves in Section 6.3.2. Storage of CB suppositories at room temperature resulted in a 2–3 ºC rightward shift of the endothermic peak (higher melting points); while a new endothermic shoulder peak was observed at approximately 38 ºC in HPKS suppositories. Both these observations lead to an increase in SFC at 37 ºC; thus preventing complete melting of the base which in turn hinders DcNa release from the base. Suppositories stored at room temperature also had lower initial rates of DcNa release, consistent with the longer softening times observed in these samples in Section 6.3.4.

Table 6.4 : The DE (%) of suppositories made using CB, CE and SS containing 50 mg DcNa and 5 %w/w bioadhesive polymer (PVP, CMCTS). Asterisks indicate a significant difference in DE compared to 'freshly prepared' suppositories. Mean ± SD, n=3.

Formulation		DE (%)				
		Freshly prepared	Refrigerated		Room temperature	
Base	Polymer		100 days	200 days	100 days	200 days
CB	PVP	90.951 ± 0.641	92.337 ± 0.957	89.299 ± 1.410	88.925 ± 0.642 *	80.546 ± 0.630 *
CE	PVP	85.829 ± 0.665	85.244 ± 1.406	87.656 ± 4.106	51.840 ± 0.651 *	56.099 ± 2.383 *
SS	PVP	86.270 ± 1.382	87.557 ± 0.862	89.823 ± 2.104 *	75.081 ± 1.344 *	79.269 ± 0.421 *
CB	CMCTS	91.673 ± 1.099	91.984 ± 1.861	85.497 ± 1.132 *	82.913 ± 0.774 *	51.139 ± 3.250 *
CE	CMCTS	92.039 ± 1.021	90.238 ± 2.207	90.379 ± 0.134	59.904 ± 4.085 *	36.582 ± 0.697 *
SS	CMCTS	91.638 ± 1.551	93.089 ± 0.236	93.620 ± 0.663	74.913 ± 1.386 *	67.064 ± 1.118 *

Table 6.5 : The MDT (minutes) of suppositories made using CB, CE and SS containing 50 mg DcNa and 5 %w/w bioadhesive polymer (PVP, CMCTS). Asterisks indicate a significant difference in MDT compared to 'freshly prepared' suppositories. Mean ± SD, n=3.

Formulation		MDT (min)				
		Freshly prepared	Refrigerated		Room temperature	
Base	Polymer		100 days	200 days	100 days	200 days
CB	PVP	24.740 ± 2.285	14.199 ± 0.835 *	28.009 ± 3.507	27.639 ± 1.839	39.763 ± 5.428 *
CE	PVP	21.478 ±1.932	18.757 ± 1.437	24.159 ± 0.955	106.426 ± 16.754 *	93.973 ± 7.685 *
SS	PVP	17.531 ± 0.511	17.369 ± 2.012	15.405 ± 2.577	42.026 ± 5.608 *	32.946 ± 3.310 *
CB	CMCTS	20.001 ± 2.660	26.794 ± 3.816	39.124 ± 2.632 *	52.453 ± 2.684 *	112.75 ± 6.897 *
CE	CMCTS	24.616 ± 3.082	27.098 ± 2.320	25.317 ± 0.424	86.192 ± 4.466 *	92.735 ± 8.642 *
SS	CMCTS	18.082 ± 3.263	16.896 ± 1.398	19.175 ± 1.055	52.315 ± 6.651 *	75.695 ± 15.255 *

6.4 Conclusion

In general, suppositories which were stored at room temperature for up to 200 days (accelerated ageing conditions) resulted in: (1) compromised aesthetic values in terms of loss of glossiness and increasing graininess; (2) increased melting point; (3) possible TAG separation suggested by the presence of new endothermic peaks; (4) higher solid fat content at 37 °C; (5) prolonged softening times; and (6) decreased rate and amount of DcNa release. Such changes to the tested formulations were unfavourable and could potentially lead to treatment failure in patients. Based on stability studies conducted in this chapter, the suppositories tested were better suited for storage in the refrigerator rather than at room temperature. There were no conclusive finding of superior stability at room temperature among HPKS and CB formulations tested as both exhibited notable changes in terms of thermal profiles and drug release after subjection to the accelerated ageing process. However, SS suppositories appear to release DcNa more efficiently than CE suppositories after being exposed to the same accelerated ageing process.

CHAPTER 7

GENERAL CONCLUSION AND FUTURE WORK

7.1 General conclusion

There has been substantial interest in utilising alternative fat sources as suppository bases due to the occurrence of up to six polymorphic forms in CB, one of the oldest lipophilic bases used in manufacturing of suppositories (Loisel et al., 1998; Marangoni and McGauley, 2003). The presence of various polymorphic forms and rigid processing requirements were a hindrance to stability and storage as well as industrial scale up. The HPKS, which have long been used in the chocolate and confectionary industry as CB substitutes in the coating of candies, caramel centrefilling and manufacturing of chocolate bars were evaluated as a potential alternative to CB for the manufacturing of suppositories.

The model drug DcNa, a nonsteroidal anti-inflammatory drug (NSAID) has been marketed for well over 30 years as oral tablets, suppositories, injectables and topical creams. Although not as popular as oral administration, suppositories may prove valuable in conditions where patients are unable to swallow their medication or are inaccessible to a qualified caregiver for parenteral administration. In fact, a recent study by van der Marel et al. (2004) found that DcNa suppositories administered in children undergoing tonsilectomy had higher relative bioavailability and needed a shorter time to achieve maximum plasma concentration compared to oral enteric coated tablets of equivalent doses.

This drug however, has been reported to undergo substantial first pass metabolism, resulting in oral bioavailability of approximately 55 % (Willis et al., 1979). Drugs administered rectally may avoid the presystemic circulation if absorbed in the lower rectum which drains into the inferior and middle hemorrhoidal veins flowing directly

into the systemic circulation, bypassing presystemic metabolism pathways (Allen et al., 2008; Kokate et al., 2006).

Therefore, this research sought to (1) evaluate HPKS as an alternative lipophilic base and (2) characterise DcNa bioadhesive suppositories produced by incorporation of bioadhesive polymers (CBP, PVP, HPMC, CMCTS) to produce suppositories which preferentially be retained at the lower rectum, thus bypassing presystemic metabolism pathways.

The two HPKS used in this study (CE and SS) were found to be suitable lipophilic suppository bases. They were comparable to CB in terms of thermal profile, SFC, pH, viscosity and DV but with added benefits of lesser polymorphic forms and less rigid manufacturing requirements. This study concluded that to manufacture CB suppositories extemporaneously, the molten base should be maintained between 34 ± 0.5 °C during heating and allowed to cool slowly to between 20–24 °C to produce suppositories with the desirable form 4A polymorph. Molten CB should not be placed into a refrigerator before solidification is complete as it will result in rapid crystallisation into the form 2 polymorph. These rigid processing restrictions however, were not relevant in HPKS. The HPKS allowed more manufacturing flexibility compared to CB.

Furthermore, the finished products (HPKS bioadhesive suppositories) were deemed suitable for rectal administration as melting points were within the range of 32.5-35.5 °C. The addition of bioadhesive polymers did not significantly alter melting point (less than ±1 °C) of the suppositories and all formulations tested contained

>95 % of the stipulated DcNa content. Softening times recorded for all the formulations were between 3–7 minutes which were acceptable for rectal drug release. Viscosity of the molten suppositories were enhanced with increasing amount of bioadhesive polymers (CBP, PVP, HPMC, CMCTS) incorporated into the formulation and this may be beneficial for retention of suppositories within the lower rectum by limiting its spread.

Apart from physical evaluation, the HPKS bases appeared to be comparable to CB in terms of drug release capacity and could be good lipophilic base candidates for fast-acting DcNa suppository formulations. Mathematical modelling of the data found that DcNa release in suppositories without polymer was via non-Fickian diffusion kinetics (Korsmeyer-Peppas model). Addition of 1-5 %w/w HPMC, PVP and CMCTS to the suppositories did not alter the mechanism of DcNa release, and these were decent candidates of polymers for development of bioadhesive suppositories. However, formulations added with 1-5 %w/w CBP significantly suppressed DcNa release compared to their respective blanks (DcNa only suppositories). The addition of CBP lead to considerable change in morphology of molten suppository during dissolution via gelling, resulting in a biphasic DcNa release process involving a rapid initial diffusion and erosion process followed by a slow diffusion process across the CBP gel layer. Furthermore, as CBP gels in the dissolution medium, it decreases the environment pH which lowers DcNa solubility, thus further retarding the release of DcNa from the suppositories.

Evaluation of bioadhesive properties of suppository formulations using the two fabricated *in vitro* methods in current research found that formulations containing

PVP exhibited superior bioadhesion compared to the other polymers when subjected to both the tensile and shear forces of detachment. This finding was promising for the development of bioadhesive suppositories. CBP displayed good bioadhesive properties only via tensile stress measurement while CMCTS showed appreciable shear stress of bioadhesion. Conversely, HPMC exhibited poor bioadhesivity in both tests and has limited role in the development of bioadhesive suppositories. Although both methods used were temperature controlled to simulate *in vivo* conditions, evaluation of shear stress of bioadhesion is more likely to reflect actual attachment and detachment of a suppository in the human rectum. Meanwhile, colon mucosa and regenerated cellulose membranes were also found to be good substitutes for rectal membranes in qualitative evaluation of bioadhesion in suppositories.

Only suppositories (CB, CE and SS) containing 5 %w/w PVP and 5 %w/w CMCTS were subjected to stability assessment, while suppositories containing 1-5 %w/w of CBP and 1-5 %w/w HPMC were omitted from further development due to their limited benefit in the formulation of bioadhesive suppositories. The concentration dependent suppression of DcNa release from CBP suppositories indicate that CBP should only be used at the lowest possible concentration but lower concentrations of CBP (1 %w/w) exhibited poor bioadhesive properties. HPMC suppositories on the other hand demonstrated poor bioadhesive properties at all concentrations tested (1-5 %w/w).

In general, suppositories stored at room temperature for up to 200 days (accelerated ageing conditions) resulted in: (1) compromised aesthetic values in terms of loss of glossiness and increasing graininess; (2) increase in melting point; (3) possible TAG

separation suggested by the presence of new endothermic peaks; (4) higher SFC at 37 °C; (5) prolonged softening times; and (6) decreased rate and amount of DcNa release. Such changes to the tested formulations were unfavourable and could potential lead to treatment failure in patients. Based on the stability studies conducted in this study, the suppositories tested were better suited for storage in refrigerator rather than at room temperature. There were no conclusive findings of formulations with superior stability at room temperature among the HPKS and CB formulations tested as both exhibited notable changes in terms of thermal profiles and drug release after subjection to the accelerated aging process. However, SS suppositories appeared to release DcNa more efficiently than CE suppositories after being exposed to the same accelerated ageing process.

Among all the formulations developed and tested, SS suppositories containing 5 %w/w PVP appeared to be the most suitable candidate for future development of bioadhesive DcNa suppositories because (1) it has less rigid manufacturing requirements compared to CB; (2) melts rapidly at human body temperature; (3) did not cause retardation of DcNa release and (4) exhibits good bioadhesive properties. However, additional work is required for *in vivo* studies to evaluate the retention capacity of the suppositories within the lower rectum as well as the actual bioavailability of these bioadhesive suppositories.

7.2 Future work

The work described in this thesis has been concerned with characterisation of two HPKS as an alternative suppository base to CB, as well as to develop bioadhesive suppositories by incorporating bioadhesive polymers into the two HPKS bases. A

number of problems and challenges were encountered during the course of this work which suggests research directions to be pursued for further development of a bioadhesive suppository system.

7.2.1 Evaluation of bioadhesive suppository migration in the rectum

The bulk of this work has been focused on the development of methods to study bioadhesion as well as evaluation of bioadhesive properties of suppository formulations. However, further studies should be designed to investigate the migration and disposition of these bioadhesive suppositories in the rectum compared to conventional suppositories. One of the methods would be to administer suppositories stained with brilliant blue to fasted Wistar rats. The rat rectums were then excised and length of the coloured regions reflected distance travelled by suppositories (Yahagi et al., 1999). An alternative method which avoids sacrificial of test subjects is by using gamma-scintigraphy. Administration of suppositories radiolabelled with technetium hydroxymethyldiphosphonate (^{99m}Tc) into volunteers would allow continuous observation of suppository migration along the human rectum via external scintigraphy (Jay et al., 1985; Sugito et al., 1988).

7.2.2 Incorporation of DcNa-polymer granules

Incorporation of bioadhesive polymers in this study has been via a direct solid dispersion in the semi-solid base. This was done to increase viscosity as well as to adhere the base matrix to the lower rectum. An alternative for further investigation would be to prepare bioadhesive granules containing drug and bioadhesive polymer (PVP and CMCTS) for incorporation into the base. These bioadhesive granules can be prepared using wet granulation methods similar to that in manufacturing of tablets

(Attama et al., 2000). This approach could improve adhesion of DcNa to the rectal mucosa by concentrating the polymers around the DcNa granules

7.2.3 Introduction of synthetic cellulose membrane as alternative to biological membranes

Current work has found that synthetic cellulose membranes were well correlated to porcine colon and rectum membranes and could be a possible alternative to biological membranes using the newly devised experimental setup for measurement of the shear forces of bioadhesion. Further investigations should be conducted using other biological and synthetic membranes to evaluate and validate this proposed correlation between biological and synthetic membranes at different experimental parameters.

7.2.4 *In vivo* bioavailability studies

This work has evaluated *in vitro* release of DcNa from the suppositories comprehensively; however, further *in vivo* evaluation using animal models or human test subjects would provide a better depiction of the systemic bioavailability of DcNa from bioadhesive suppositories. The pharmacokinetics and metabolism of DcNa in Yucatan miniature pigs (Oberle et al., 1994) and rats (Reiss et al., 1978) were similar to man. While avoidance of first pass metabolism via the rectal route was demonstrated in rabbits (Kurosawa et al., 1998) and rats (De Leede et al., 1983), where bioavailability of drugs increased as the site of absorption was closer to the anus. These observations were similar to that observed in man. Therefore, rats would be a reasonable model for *in vivo* studies. Aoyagi et al. (1988) studied bioavailability of indomethacin suppository in rabbits and pigs by administering the suppository to the fasted test subjects and plasma samples were withdrawn for analysis at specific

intervals. Comparison of DcNa bioavailability between bioadhesive and conventional suppositories would provide an indication of effectiveness in avoidance of first pass metabolism provided by the addition of bioadhesive polymers.

There is clearly much work to be done in the development of a bioadhesive suppository system. Perhaps, the most direct extension to this work is the evaluation of bioadhesive suppository migration in the rectum post administration. Comparison of shortlisted formulations from this work against conventional suppositories would elucidate feasibility and practicality of a bioadhesive suppository.

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APPENDIX

Appendix 1: Certificate of analysis of cocoa butter (CB).

JBCOCOA

JB COCOA SDN BHD
(514587-A)
LOT CP1, JALAN TANJUNG A/6,
PELABUHAN TANJUNG PELEPAS,
81560 GELANG PATAH, JOHOR,
MALAYSIA.
Email: info@jbcocoa.com.my
TEL : (6)07-504 2888
FAX : (6)07-507 1388
Website: www.jbcocoa.com

Certificate of Analysis

Customer	: School of Pharmacy	**Container No.**
Date	: 17/Jan/12	-
Product Type	: Pure Prime Pressed Cocoa Butter	
Product Code	: JB100-PPP	
Lot No.	: 121085	
Prod Date	: 08/Jan/12	
Expiry Date	: 2 years from date of manufacturing	**Product Weight/Count:**
Invoice No	: 3004199	1 X 25.00 KG

Control Parameter	Product Specification	Test Results
		121085
Physical Characteristic		
Free Fatty Acid (%)	1.75 Max	1.68
Iodine value	32-38	36.89
Moisture (%)	0.30 Max	0.01
Microbiological Characteristic		
Total Plate Count per Gram	1,000 Max	<100
Yeast & Mold per Gram	50 Max	<10
Enterobacteriaceae in 1 g	Not detected	Not Detected
Coliform in 1 g	Not detected	Not Detected
Eschericia coli in 1 g	Not detected	Not Detected
Salmonella in 375 g	Not detected	Not Detected

These test result apply to an average sample taken from the goods when they leave the production plant. They are analyzed according to the methods of analysis as described in in-house testing method.

Reported by,

QA/QC Executive

Verified by,

QA Manager/
Asst.QA Manager

Appendix 2 : Certificate of analysis for Chocexa (CE).

LAM SOON EDIBLE OILS SDN. BHD. (Co. No.: 14578-T)
Lot 10, Jalan Timah, P. O. Box 70, 81707 Pasir Gudang, Johore, Malaysia.
Tel: +60-7-2512 101 Fax: +60-7-2525 696

Date : 16.08.2010

To Whom It May Concern:

CERTIFICATE OF ANALYSIS

Product / Sample : ChocExa

Packing : 1 X 20kg

Analysis Results:

Free Fatty Acid (as Lauric)	0.02 %
Colour (5.25" Lovibond Cell)	Red 0.1
Moisture & Impurities	0.04 %
Iodine Value (wijs)	0.4
Saponification Value	250
Slip Melting Point (AOCS Cc 3-25)	35.0°C
Solid Fat Content (N-Value)	
20°C	95.8 %
30°C	49.1 %
35°C	4.4 %

For Lam Soon Edible Oils Sdn. Bhd.

LE Loh

LE Loh
Food Technologist
Quality Management and Research Department

Appendix 3 : Certificate of analysis for Supersocolate Special™ (SS).

Cargill

CERTIFICATE OF ANALYSES

DATE : Jan 13th 2011 REPORT NO : S11-012

COMPANY : **University of Nottingham Malaysia Campus**
School of Pharmacy
Jalan Broga, 43500 Semenyih, Selangor

ATTN : **Dr Tung Wai Hau**

PRODUCT / BRAND : **SUPER SOCOLATE SPECIAL** QUANTITY : 1 X 2KG
GENERIC NAME : **RBD HYDROGENATED PK STEARIN**
BATCH / TANK NO : 10100120021

ANALYSES RESULTS.

PARAMETERS	METHOD	SPECS.	RESULTS
FREE FATTY ACIDS (AS % C12)	A.O.C.S. Ca 5a-40	0.1 Max	0.023
IODINE VALUE (WIJ'S METHOD)	A.O.C.S. Cd 1b-92	1.0 Max	0.29
COLOR (5.25" LOVIBOND CELL)	PORIM P4.1	1.0 Red Max	0.6 Red
MOISTURE & IMPURITIES (%)	A.O.C.S. Ca 2b-38	0.1 Max	0.029
SLIP MELTING POINT (DEG C)	A.O.C.S. Cc 3 -25	33.5 - 35.5	34.6
FLAVOUR	SENSORY	BLAND	Typical
ODOUR	SENSORY	BLAND	Typical
DIRT TEST	FILTER TEST	GRADE 7	Passed

Cargill Palm Products Sdn. Bhd.
(45493-W)

Lab QC

Cargill Palm Products Sdn. Bhd. (Reg. No. 45493-W)
167, Jalan Kem, 42000 Port Klang, Selangor Darul Ehsan, Malaysia
Tel : +603 3165 3888
Fax : +603 3168 6546

Appendix 4 : Certificate of analysis for diclofenac sodium (DcNa).

SHREEJI
PHARMA INTERNATIONAL

FF/10, Narsinghdham Complex,
Harni Air Port Road, Vadodara-390022. (INDIA)
Telefax : +91-265-2465036. Ph. : +91-265-3019110
E-mail : info@shreejipharma.com
Website : www.shreejipharma.com

Certificate of Analysis

Product : Diclofenac Sodium IP **Batch No : 184/10**

Mfg Date : Feb – 2010 **Exp Date : Jan – 2015**

Tests	Specification	Results
Description	White to slight yellowish crystalline powder, slightly hygroscopic	White crystalline powder
Solubility	Freely soluble in Methanol & Ethanol, sparingly soluble in water	Complies
Melting range	About 280°C with Decomposition	279°C-281°C with Decomposition
Identification	By IR	Complies
pH	Between 6.5 to 8.5	Complies
Light Absorbance at 440nm	Not more than 0.05	0.02
Heavy Metals	Not more than 10ppm	Complies
Loss on Drying	Not more than 0.5%	0.13%W/W
Related substances	Total Impurities not more than 1.0%	Complies
Assay	Not less than 99% & not more than 101.0% of $C_{14}H_{10}Cl_2NNaO_2$ calculated with reff. to the dried substances.	99.85

SHREEJI PHARMA INTERNATIONAL
INDIA

Appendix 5 : Certificate of analysis for Carbopol 974P NF (CBP).

Lubrizol

Product Specification

CARBOPOL®* 974P NF POLYMER

Carbopol® 974P NF polymer meets the limits cited in the current edition of the following monographs:

- United States Pharmacopeia/National Formulary (USP/NF) monograph for Carbomer Homopolymer Type B
  (Note: The previous USP/NF compendial name for this product was Carbomer 934P.)
- European Pharmacopeia (Ph. Eur.) monograph for Carbomers

Applicable synonyms for Carbopol® 974P NF polymer are carboxypolymethylene and carbomers.

General Product Characteristics
Appearance: White, fluffy powder
Odor: Slightly acetic

Test	Specification	Lot Test Frequency[1]	Test Procedure[2]
Identification			
Colorimetric test	Pass	1:200	USP/NF
Gel formation test	Pass	1:200[3]	USP/NF
Infrared spectrum	Pass	---[4]	Lubrizol SA-102
Precipitate test	Pass	1:200	USP/NF
Carboxylic Acid Content, Assay %	56.0 - 68.0	1:1	Lubrizol 1318-A
Viscosity, cP, 25°C			
Brookfield RVT, 20 rpm, neutralized to pH 7.3 - 7.8			
0.5 wt% mucilage, spindle #6	29,400 - 39,400	1:1	Lubrizol 430-I
Loss on Drying, %	2.0 max	1:1	USP/NF
Heavy Metals, ppm			
Total heavy metals, as Pb	20 max	1:200	USP/NF
Specific metals: Hg, Pb, As, Sb	10 max	1:200	Lubrizol SA-012
Residual Solvent[5]			
Ethyl acetate, %	0.50 max	1:1	Lubrizol SA-009
Benene[6]			
Benzene, ppm	0.50 max	1:1	Lubrizol SA-064
Residual Monomer, ppm			
Free acrylic acid	1,000 max	1:1	Lubrizol SA-005
Sulphated Ash, % (Residue on Ignition)	2.5 max	1:200	USP/NF

[1] Where lot test frequency is less than 1:1, Lubrizol Advanced Materials, Inc. certifies that each batch/lot meets requirements for the characteristics based on historical process and product data. Because these characteristics are tested on a skip-lot test frequency, results are not reported on the Certificate of Analysis.

[2] Lubrizol test procedures have been cross-validated to specified compendial procedure(s) or validated if they are included in the monograph.

[3] Gel formation is confirmed by the viscosity test procedure (Lubrizol 430-I) for each lot of polymer that is produced. Every 200 lots, the gel formation test is conducted according to USP requirements.

[4] Infrared reference spectra available upon request.

[5] No other residual solvents as listed in USP/NF <467> (Class 1, 2, 3, Table 4 or any other solvents) or Ph. Eur. 2.4.24 are used in the manufacturing process of this product.

[6] Benzene is tested due to it being a potential impurity.

Edition: September 9, 2011
Revisions: February 6, 2008 / August 9, 2010 / May 25, 2011
Issue date: June 14, 2007

Appendix 6 : Certificate of analysis of hydroxypropyl methylcellulose 2910 (HPMC).

NEWSTAR CHEM ENTERPRISE LTD

CERTIFICATE OF ANALYSIS

Product:	Hydroxypropyl Methyl Cellulose		
Quantity:	1kg	Date of manufacture:	2011-12-12
		Date of report:	2011-12-15
Lot number:	201112121	Date of expiry	2014-12-11

TEST ITEM	UNIT	SPECIFICATION	TEST RESULT
APPEARANCE		White to slightly off-white fibrous or granular powder.	White powder.
IDENTIFICATION A TO E		Conform	Conform
SOLUTION APPEARANCE		Conform	Conform
METHOXY	WT%	28.0-30.0	28.4
HYDROXYPROPOXY	WT%	7.0-12.0	7.6
LOSS ON DRYING	WT%	≤5.0	3.2
RESIDUE ON IGNITION	WT%	≤1.5	1.0
PH		5.0-8.0	6.7
HEAVY METAL	PPM	≤10	<10
LEAD	PPM	≤3	<3
ARSENIC	PPM	≤3	<3
MERCURY	PPM	≤1	<1
CADMIUM	PPM	≤1	<1
TOTAL PLATE COUNT		≤1000	<1000
YEAST AND MOULD		≤100	<100
COLI FORM		Absent	Absent
SALMONELLA		Absent	Absent
RESIDUAL SOLVENTS		Absent	Absent
PARTICLE SIZE		98% pass through 100 mesh	Conform

This material meets all requirements of USP31 for the monograph Hydroxypropyl Methyl Cellulose 2910.

Auditing: 王静莉 Analyse: 吕坤、王兰芳

Appendix 7 : Certificate of analysis for carboxymethyl chitosan (CMCTS).

China·Eastar Holding Group (Dong Chen) Co., Ltd.

Shanghai Office: Room 2116, Lianhe Tower, No. 350 Wuning Road, Putuo District, Shanghai, China-200063
Tel: 0086-21-51275653 Email: postmaster@chinaeastargroupsh.com
Fax: 0086-21-51275655 Web Site: www.chinaeastargroupsh.com

Certificate of Analysis

Product Name	Carboxymethyl Chitosan (Water Soluble)		
Manufacture Date	2011.10.26	Quantity	3 kg
Batch Number	DCSHH-111026	Analysis Date	2011.11.06

ITEM	STANDARD	TESTING RESULT
Appearance	Off-white or light yellow powder	Complied
Degree of Substitution %	≥80%	81.9%
Viscosity(1%@20℃)	≤100mpa.s	22mpa.s
Moisture	≤15.00%	12.2%
Ash	≤1%	0.86%
DAC Degree	≥95.00%	96.5%
Arsenic	≤0.5ppm	<0.5ppm
Heavy metals	≤20ppm	<10ppm
pH(1%)	7.0-9.0	7.9
Particle Size	95%Through 80 Mesh Sieve	Complied
Total Plate Count	≤5000cfu/g	<1000cfu/g
Molds &Yeast	≤100cfu/g	<100cfu/g
E.Coli	Negative	Negative
Salmonella	Negative	Negative

Appendix 8 : The viscosity (cp) of CB suppositories measured at 50 rpm shear rate. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	54.6 ± 4.14	54.6 ± 4.14	54.6 ± 4.14	54.6 ± 4.14
DcNa only (B)	82.8 ± 1.29	82.8 ± 1.29	82.8 ± 1.29	82.8 ± 1.29
1 %w/w polymer (C)	78.43 ± 0.95	90.03 ± 2.95	84.83 ± 2.53	94.23 ± 2.58
2 %w/w polymer (D)	76.03 ± 0.76	104.8 ± 3.33	92.30 ± 0.40	98.73 ± 4.65
5 %w/w polymer (E)	87.87 ± 6.27	122.3 ± 1.06	97.67 ± 2.27	106.6 ± 0.92
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E C & E	D & E	A & B A & C A & D A & E B & D	B & E C & D C & E D & E	A & B A & C A & D A & E B & D	B & E C & E	A & B A & C A & D A & E B & C	B & D B & E C & E

Appendix 9 : The viscosity (cp) of CE suppositories measured at 50 rpm shear rate. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	53.43 ± 4.74	53.43 ± 4.74	53.43 ± 4.74	53.43 ± 4.74
DcNa only (B)	42.00 ± 0.96	42.00 ± 0.96	42.00 ± 0.96	42.00 ± 0.96
1 %w/w polymer (C)	68.10 ± 2.08	46.20 ± 1.79	43.70 ± 2.02	41.70 ± 1.87
2 %w/w polymer (D)	79.33 ± 1.15	56.10 ± 1.06	44.17 ± 1.27	49.93 ± 2.12
5 %w/w polymer (E)	102.60 ± 6.52	67.77 ± 3.10	56.40 ± 4.06	52.47 ± 5.24
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C	B & D B & E C & D C & E D & E	A & B A & E B & D B & E C & D	C & E D & E	A & B A & C B & E C & E D &E	A & B A & C B & E C & E

Appendix 10 : The viscosity (cp) of SS suppositories measured at 50 rpm shear rate. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	49.03 ± 1.46	49.03 ± 1.46	49.03 ± 1.46	49.03 ± 1.46
DcNa only (B)	37.73 ± 1.06	37.73 ± 1.06	37.73 ± 1.06	37.73 ± 1.06
1 %w/w polymer (C)	44.33 ± 1.89	41.40 ± 0.53	39.00 ± 1.52	40.73 ± 0.45
2 %w/w polymer (D)	55.87 ± 1.70	48.83 ± 0.80	44.93 ± 1.00	41.30 ± 0.91
5 %w/w polymer (E)	81.8 ± 4.78	67.77 ± 3.10	56.50 ± 2.23	46.15 ± 1.91
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & D A & E B & C B & D	B & E C & D C & E D & E	A & B A & C A & E B & D B & E	C & D C & E D & E	A & B A & C A & D B & E	

Appendix 11 : The hardness (N) of CB suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	59.00 ± 2.97	59.00 ± 2.97	59.00 ± 2.97	59.00 ± 2.97
DcNa only (B)	73.33 ± 2.07	73.33 ± 2.07	73.33 ± 2.07	73.33 ± 2.07
1 %w/w polymer (C)	100.67 ± 4.27	101.00 ± 4.56	89.00 ± 1.90	121.50 ± 2.43
2 %w/w polymer (D)	91.67 ± 5.28	92.50 ± 1.76	103.00 ± 1.41	116.67 ± 2.16
5 %w/w polymer (E)	100.67 ± 3.01	80.83 ± 4.36	99.83 ± 1.47	131.17 ± 2.32
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C B & D B & E C & D D & E	A & B A & C A & D A & E B & C B & D B & E C & D D & E	A & B A & C A & D A & E B & C B & D B & E C & D D & E	A & B A & C A & D A & E B & C B & D B & E C & E D & E

Appendix 12 : The hardness (N) of CE suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION				
	CBP	**HPMC**	**PVP**	**CMCTS**	
Blank (A)	82.33 ± 5.20	82.33 ± 5.20	82.33 ± 5.20	82.33 ± 5.20	
DcNa only (B)	88.33 ± 2.34	88.33 ± 2.34	88.33 ± 2.34	88.33 ± 2.34	
1 %w/w polymer (C)	89.00 ± 3.41	2.64 ± 2.64	99.33 ± 5.09	104.83 ± 7.57	
2 %w/w polymer (D)	84.17 ± 5.85	83.83 ± 4.17	94.17± 2.79	122.00 ± 6.99	
5 %w/w polymer (E)	103.50 ± 2.35	87.83 ± 4.22	91.33 ± 3.93	106.67 ± 8.09	
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B B & E C & E D & E	B & C	A & C A & D B & C	A & C A & D A & E B & C B & D	B & E C & D D & E

Appendix 13 : The hardness (N) of SS suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	55.83 ± 1.60	55.83 ± 1.60	55.83 ± 1.60	55.83 ± 1.60
DcNa only (B)	70.17 ± 1.72	70.17 ± 1.72	70.17 ± 1.72	70.17 ± 1.72
1 %w/w polymer (C)	103.50 ± 2.88	83.00 ± 4.20	91.17 ± 2.48	100.00 ± 5.18
2 %w/w polymer (D)	98.33 ± 4.50	70.33 ± 5.85	96.00 ± 1.55	102.50 ± 5.32
5 %w/w polymer (E)	74.67 ± 4.97	69.83 ± 2.76	90.50 ± 1.87	101.50 ± 5.65
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C B & D C & E D & E	A & B A & C A & D A & E B & C C & D C & E	A & B A & C A & D A & E B & C B & D B & E	A & B A & C A & D A & E B & C B & D B & E

Appendix 14 : The softening time (min) of CB suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	3.03 ± 0.01	3.03 ± 0.01	3.03 ± 0.01	3.03 ± 0.01
DcNa only (B)	3.79 ± 0.11	3.79 ± 0.11	3.79 ± 0.11	3.79 ± 0.11
1 %w/w polymer (C)	4.06 ± 0.03	3.82 ± 0.126	3.83 ± 0.106	3.68 ± 0.202
2 %w/w polymer (D)	3.68 ± 0.09	4.08 ± 0.051	4.26 ± 0.025	3.77 ± 0.035
5 %w/w polymer (E)	4.16 ± 0.04	4.31 ± 0.054	4.03 ± 0.017	4.20 ± 0.033
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C B & E C & D D & E	A & B A & C A & D A & E B & D B & E C & D C & E	A & B A & C A & D A & E B & D C & D	A & B A & C A & D A & E B & E C & E D & E

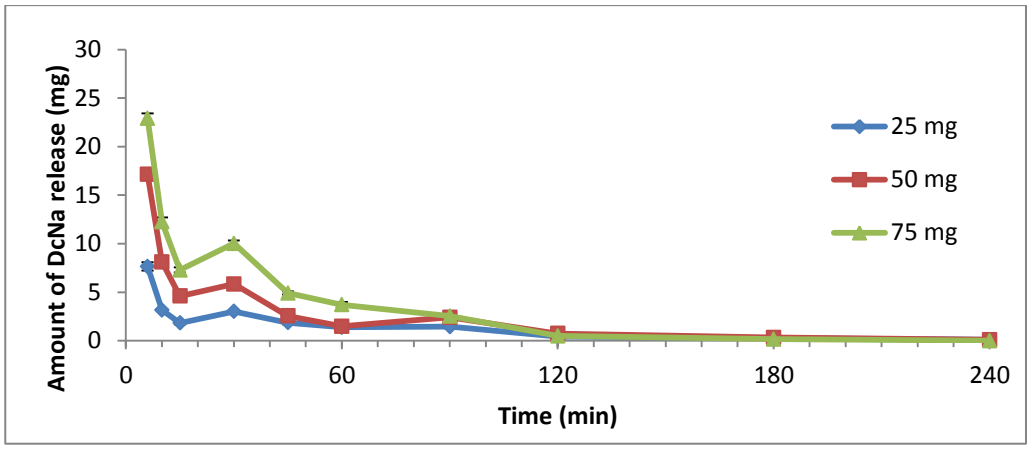
Appendix 15 : The softening time (min) of CE suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	6.16 ± 0.17	6.16 ± 0.17	6.16 ± 0.17	6.16 ± 0.17
DcNa only (B)	4.36 ± 0.07	4.36 ± 0.07	4.36 ± 0.07	4.36 ± 0.07
1 %w/w polymer (C)	4.60 ± 0.09	4.79 ± 0.03	4.66 ± 0.06	4.92 ± 0.09
2 %w/w polymer (D)	5.68 ± 0.09	5.07 ± 0.04	5.07 ± 0.05	4.99 ± 0.13
5 %w/w polymer (E)	5.34 ± 0.08	6.84 ± 0.08	5.58 ± 0.08	4.92 ± 0.08
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & D B & E C & D C & E D & E	A & B A & C A & D A & E B & C B & D B & E C & D C & E D & E	A & B A & C A & D A & E B & C B & D C & D C & E D & E	A & B A & C A & D A & E B & C B & D B & E

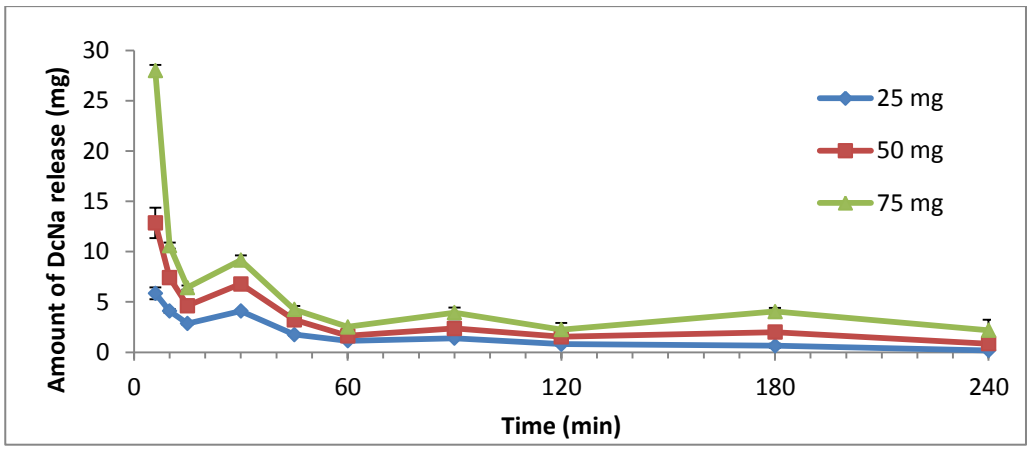
Appendix 16 : The softening time (min) of SS suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
Blank (A)	5.66 ± 0.10	5.66 ± 0.10	5.66 ± 0.10	5.66 ± 0.10
DcNa only (B)	4.36 ± 0.13	4.36 ± 0.13	4.36 ± 0.13	4.36 ± 0.13
1 %w/w polymer (C)	4.48 ± 0.13	4.81 ± 0.08	4.56 ± 0.06	4.93 ± 0.03
2 %w/w polymer (D)	4.72 ± 0.05	5.16 ± 0.05	4.76 ± 0.04	4.76 ± 0.09
5 %w/w polymer (E)	5.43 ± 0.04	6.14 ± 0.04	5.13 ± 0.04	5.29 ± 0.04
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & D	B & E C & D C & E D & E	A & B A & C A & D A & E B &C	B & D B & E C & D C & E D & E	A & B A & C A & D A & E B &C	B & D B & E C & D C & E D & E	A & B A & C A & D A & E B &C	B & D B & E C & E D & E

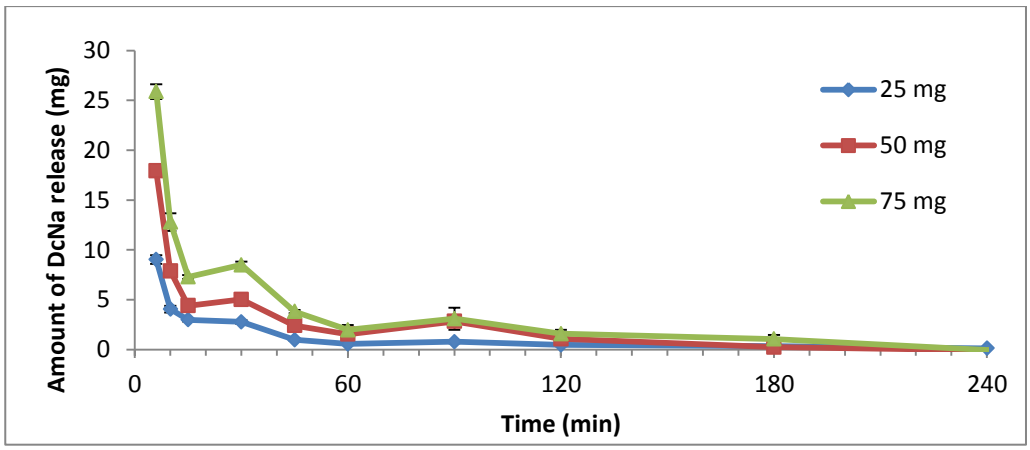
(a)



(b)

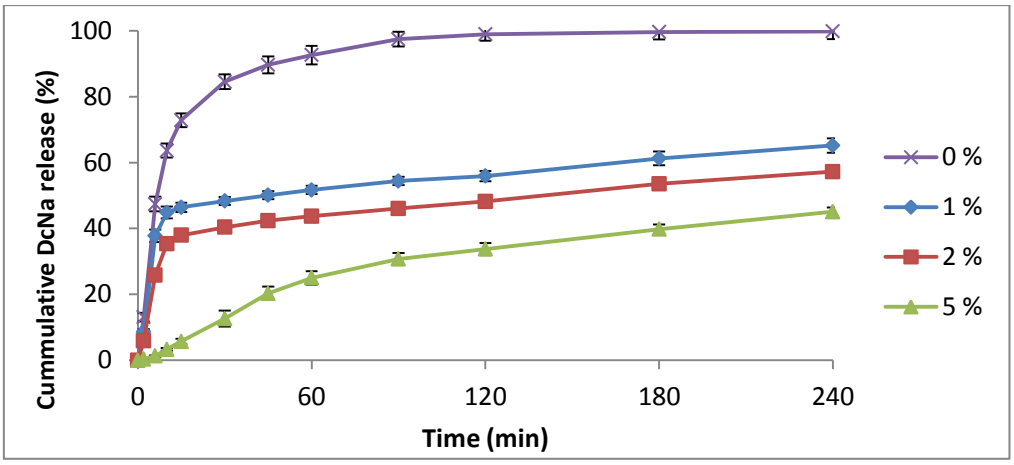


(c)

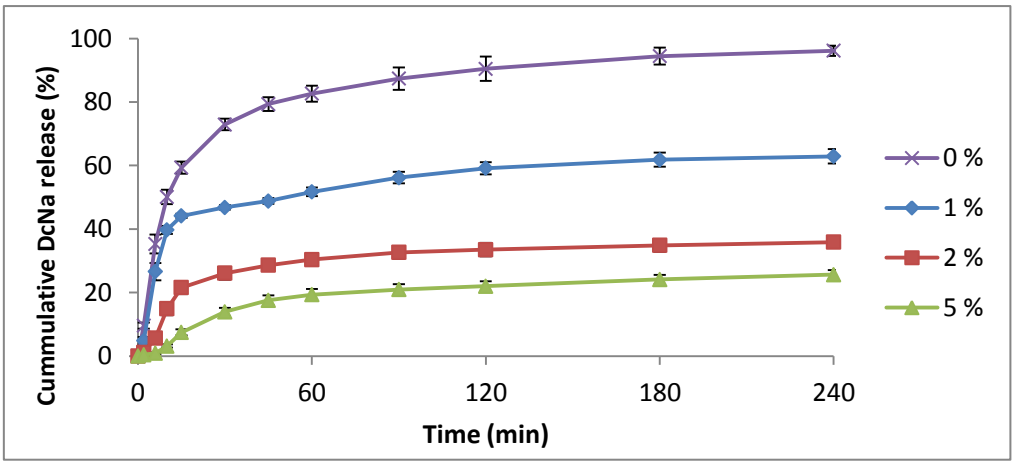


Appendix 17: Amount of DcNa (mg) released at each time interval in (a) CB; (b) CE; and (c) SS suppositories containing 25, 50, 75 mg of DcNa. Mean ± 2 SE, n=6.

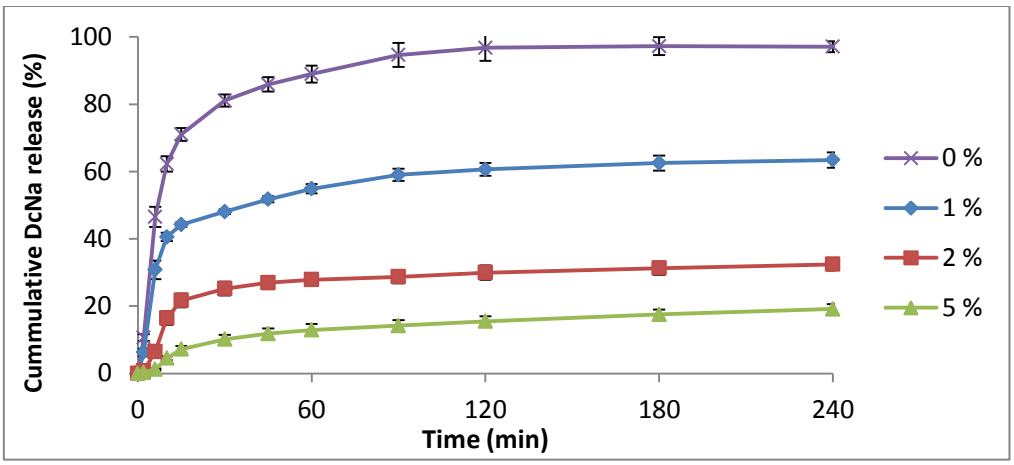
a)



b)

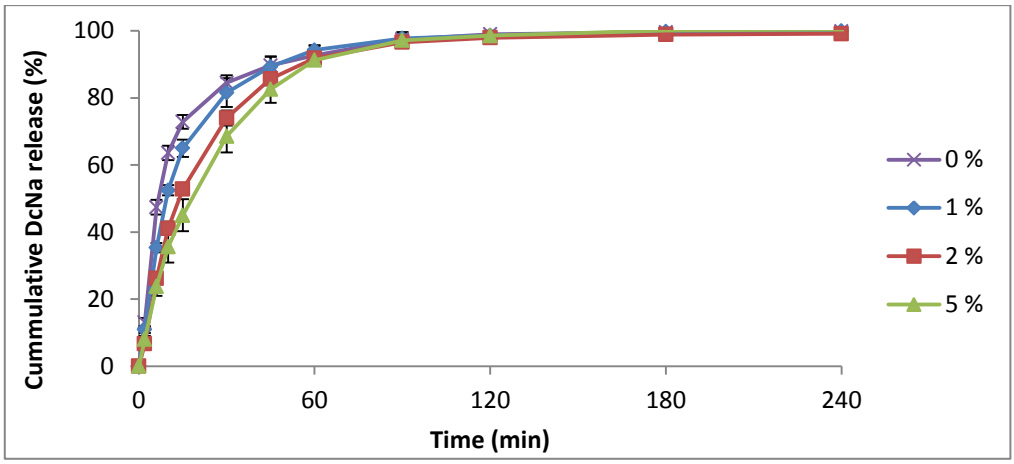


c)

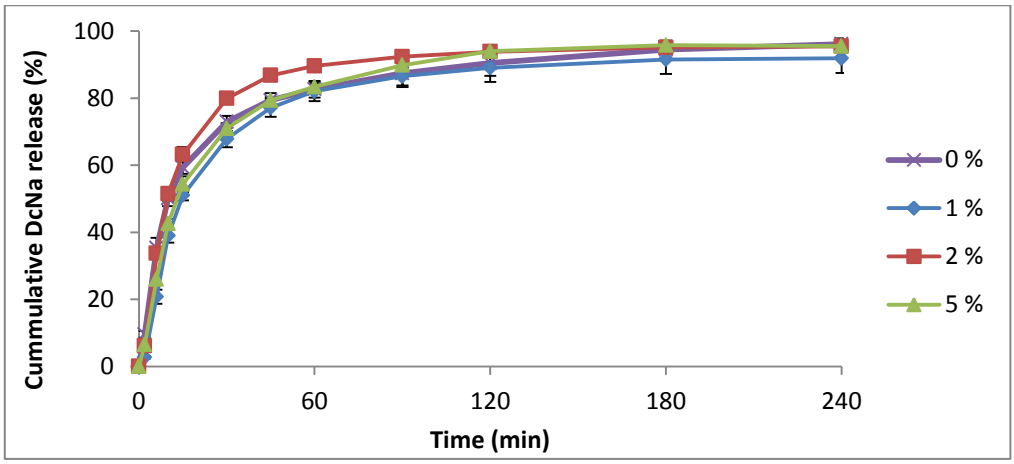


Appendix 18: Cumulative percentage DcNa release in a) CB; b) CE; c) SS suppositories incorporated with 1-5 %w/w of CBP. Mean ± 2 SE, n=6.

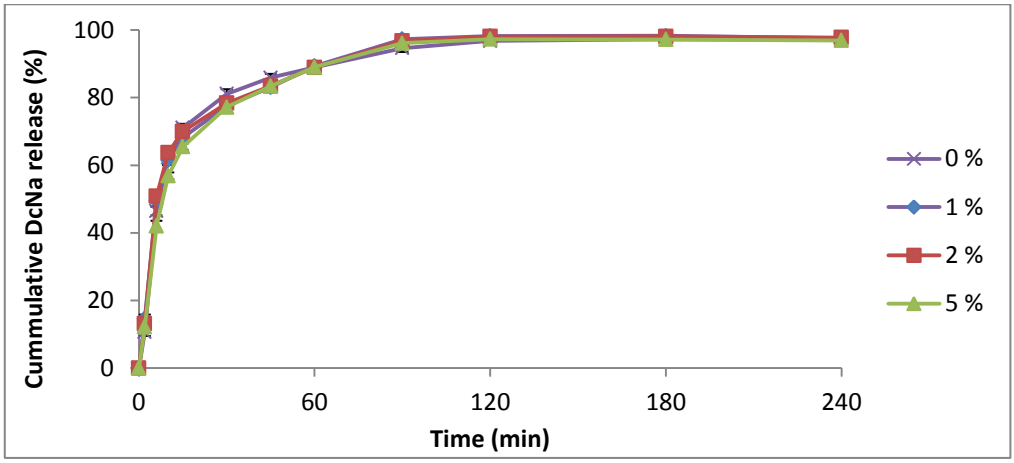
a)



b)

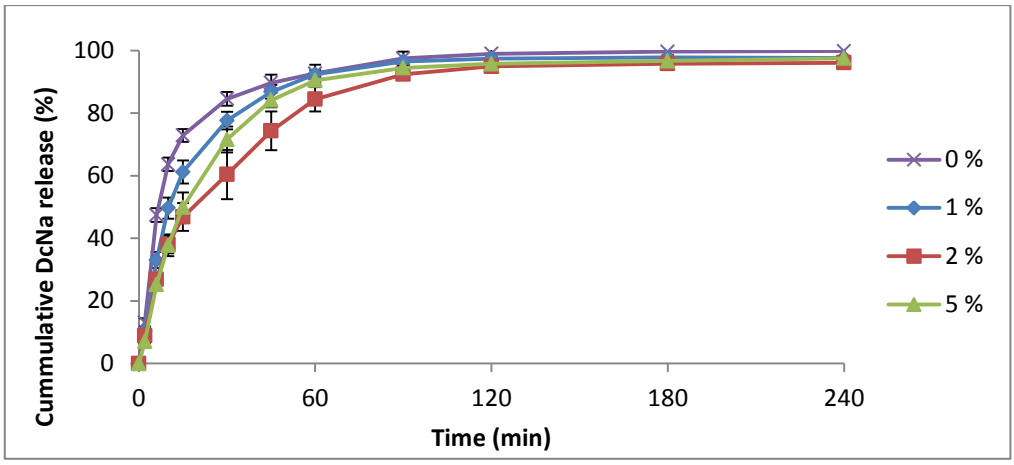


c)

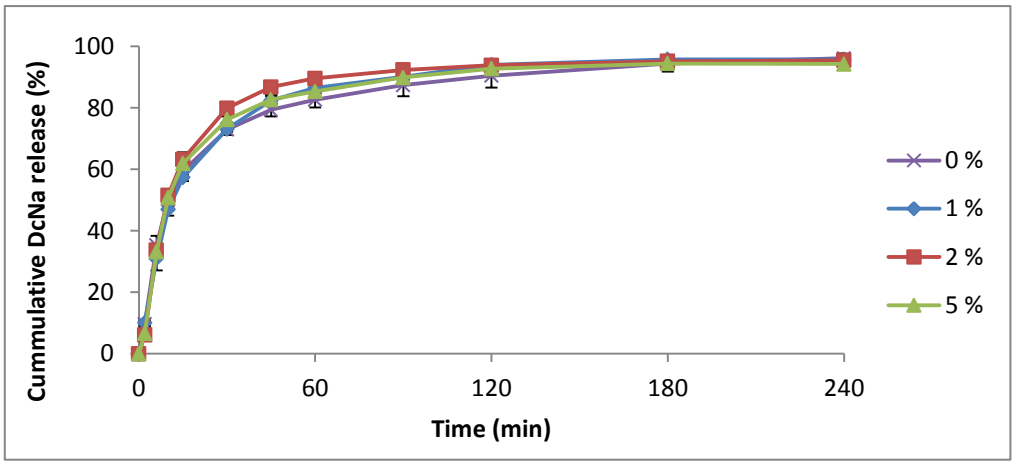


Appendix 19 : Cumulative percentage of DcNa release in a) CB; b) CE; c) SS suppositories incorporated with 1-5 %w/w of HPMC. Mean ± 2 SE, n= 6.

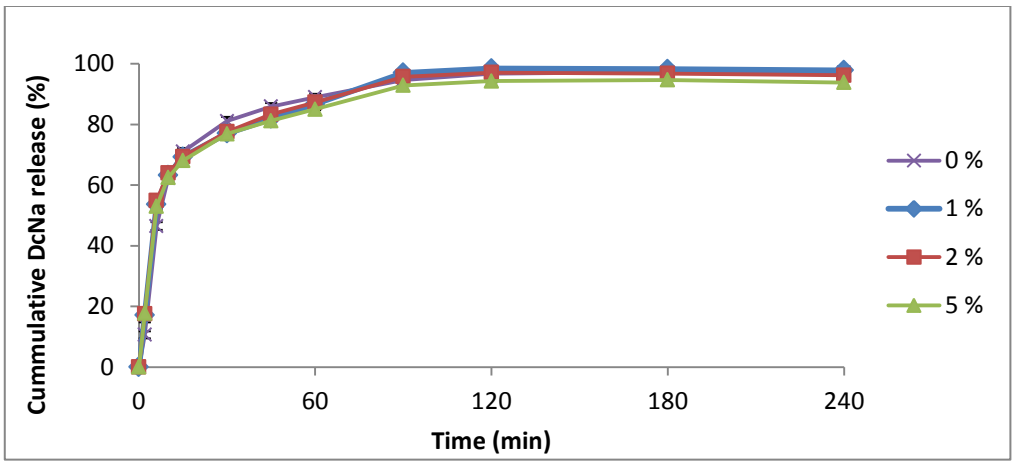
a)



b)

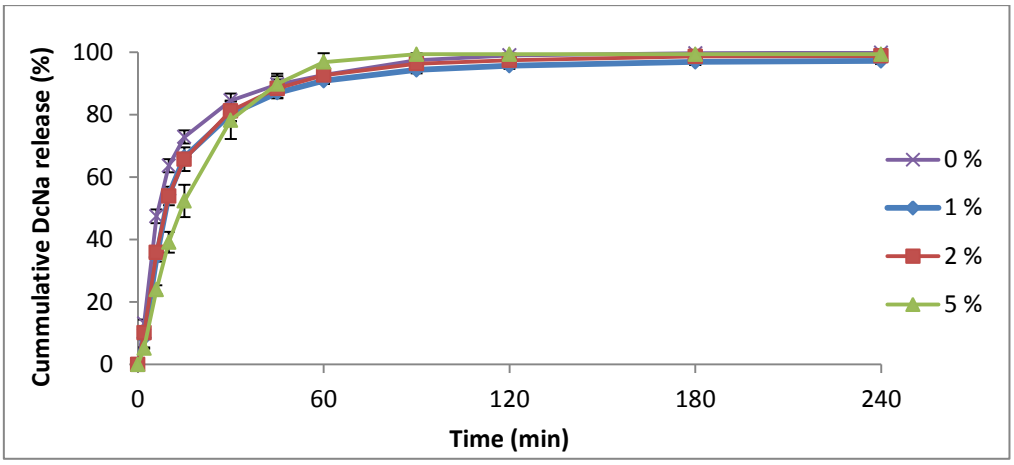


c)

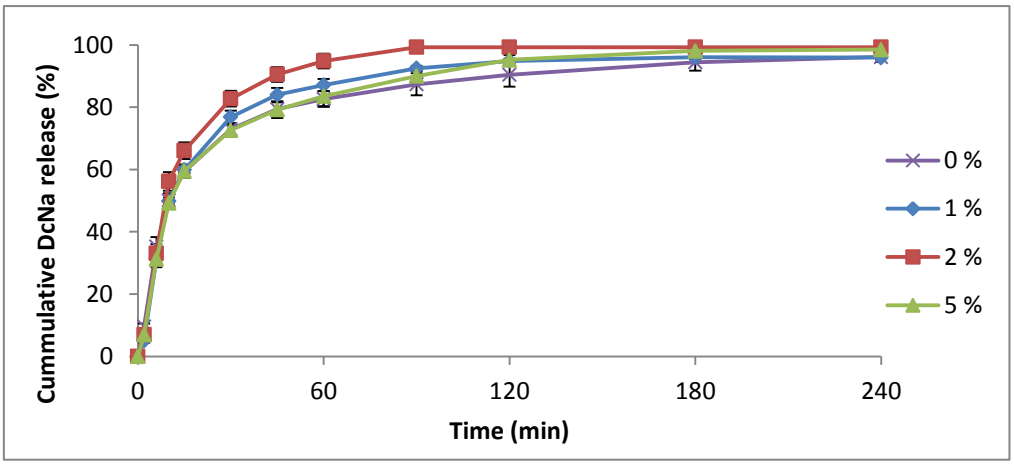


Appendix 20 : Cumulative percentage of DcNa release in a) CB; b) CE; c) SS suppositories incorporated with 1-5%w/w PVP. Mean ± 2 SE, n=6.

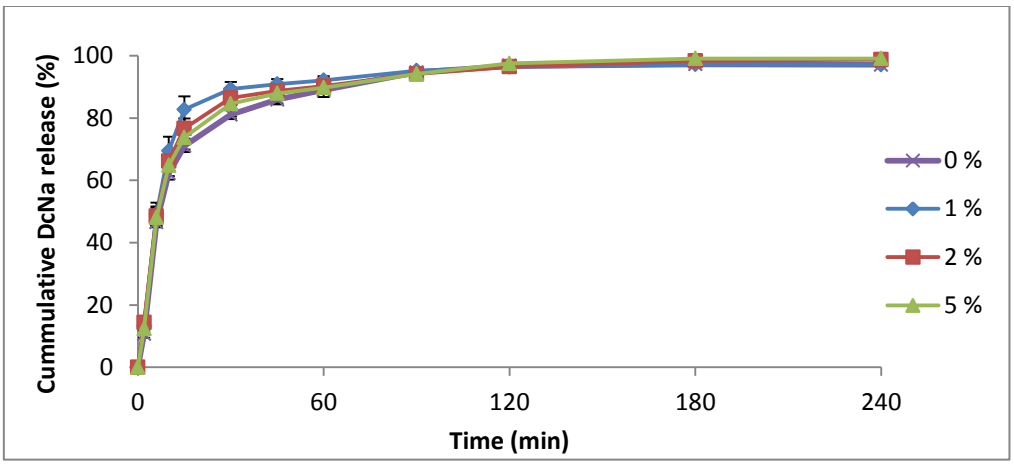
a)



b)



c)



Appendix 21 : Cumulative percentage of DcNa release in a) CB; b) CE; c) SS suppositories incorporated with 1-5%w/w CMCTS. Mean ± 2 SE, n=6.

Appendix 22 : The dissolution efficiency (DE) of CB suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	93.79 ± 1.52	93.79 ± 1.52	93.79 ± 1.52	93.79 ± 1.52
1 %w/w polymer (B)	59.65 ± 1.74	94.27 ± 1.18	92.56 ± 0.76	89.52 ± 0.95
2 %w/w polymer (C)	51.78 ± 1.34	92.87 ± 0.88	88.83 ± 1.22	90.97 ± 2.19
5 %w/w polymer (D)	36.80 ± 1.14	92.68 ± 0.83	90.95 ± 0.64	91.67 ± 1.10
ANOVA	P < 0.05	P > 0.05 (0.078)	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D B & C B & D	C & D	A & C A & D B & C	A & B A & C A & D

Appendix 23 : The dissolution efficiency (DE) of CE suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	88.73 ± 2.12	88.73 ± 2.12	88.73 ± 2.12	88.73 ± 2.12
1 %w/w polymer (B)	58.47 ± 1.56	83.55 ± 2.32	86.55 ± 0.60	87.62 ± 0.65
2 %w/w polymer (C)	33.20 ± 1.81	87.58 ± 1.03	87.91 ± 0.51	91.74 ± 1.14
5 %w/w polymer (D)	22.59 ± 1.37	85.52 ± 0.64	85.83 ± 0.67	92.04 ± 1.02
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D B & C B & D C & D	A & B A & D B & C B & D	A & D	A & C A & D B & C B & D

Appendix 24 : The dissolution efficiency (DE) of SS suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	91.93 ± 1.04	91.93 ± 1.04	91.93 ± 1.04	91.93 ± 1.04
1 %w/w polymer (B)	59.29 ± 1.71	90.76 ± 0.37	88.13 ± 0.37	92.04 ± 1.02
2 %w/w polymer (C)	29.91 ± 1.91	90.78 ± 1.17	87.62 ± 0.67	91.60 ± 0.95
5 %w/w polymer (D)	16.49 ± 2.56	89.70 ± 0.53	86.27 ± 1.38	91.64 ± 1.55
ANOVA	P < 0.05	P < 0.05	P < 0.05	P > 0.05 (0.892)
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D B & C B & D	C & D	A & D	A & B A & C A & D

Appendix 25 : The min dissolution time (MDT) of CB suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	14.73 ± 2.47	14.73 ± 2.47	14.73 ± 2.47	14.73 ± 2.47
1 %w/w polymer (B)	60.72 ± 2.09	19.04 ± 2.28	18.21 ± 1.60	18.89 ± 0.98
2 %w/w polymer (C)	69.24 ± 2.09	24.08 ± 1.78	27.98 ± 3.85	18.47 ± 2.14
5 %w/w polymer (D)	114.03 ± 4.42	26.25 ± 2.80	24.74 ± 2.28	20.03 ± 2.66
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D B & D C & D B & C	A & C A & D B & C B & D	A & C A & D B & C B & D	A & D

Appendix 26 : The min dissolution time (MDT) of CE suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	26.44 ± 3.32	26.44 ± 3.32	26.44 ± 3.32	26.44 ± 3.32
1 %w/w polymer (B)	36.36 ± 1.73	26.07 ± 2.12	23.17 ± 1.28	20.81 ± 1.91
2 %w/w polymer (C)	43.04 ± 3.13	19.85 ± 1.48	19.01 ± 1.08	17.34 ± 1.91
5 %w/w polymer (D)	74.15 ± 6.14	25.04 ± 0.95	21.48 ± 1.93	24.62 ± 3.08
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D B & C B & D C & D	A & C B & C C & D	A & C A & D	A & B A & C C & D

Appendix 27 : The min dissolution time (MDT) of SS suppositories. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
0 %w/w polymer (A)	15.60 ± 1.07	15.60 ± 1.07	15.60 ± 1.07	15.60 ± 1.07
1 %w/w polymer (B)	30.63 ± 2.89	16.77 ± 0.50	18.49 ± 0.19	14.82 ± 3.00
2 %w/w polymer (C)	42.18 ± 7.35	17.15 ± 0.81	17.03 ± 1.28	17.43 ± 2.44
5 %w/w polymer (D)	83.01 ± 10.39	17.92 ± 0.52	17.53 ± 0.51	18.08 ± 3.26
ANOVA	P < 0.05	P < 0.05	P < 0.05	P > 0.05 (0.134)
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B C & D A & C A & D B & C B & D	A & D	A & B A & C A & D	

Appendix 28 : The peak force of detachment (F_{max}) of CB suppositories measured using tensile setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	2.17 ± 0.43	2.17 ± 0.43	2.17 ± 0.43	2.17 ± 0.43
1 %w/w polymer (B)	2.48 ± 0.43	2.19 ± 0.47	2.74 ± 0.55	2.51 ± 0.32
2 %w/w polymer (C)	3.04 ± 0.46	2.65 ± 0.40	3.07 ± 0.48	2.93 ± 0.44
5 %w/w polymer (D)	4.17 ± 0.74	2.74 ± 0.70	3.89 ± 0.38	3.52 ± 0.47
ANOVA	P < 0.05	P > 0.05 (0.40)	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & D		A & D B & D C & D	A & D B & D

Appendix 29 : The peak force of detachment (F_{max}) of CE suppositories measured using tensile setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	2.12 ± 0.30	2.12 ± 0.30	2.12 ± 0.30	2.12 ± 0.30
1 %w/w polymer (B)	3.28 ± 0.41	2.18 ± 0.46	2.81 ± 0.54	3.00 ± 0.40
2 %w/w polymer (C)	3.08 ± 0.40	2.39 ± 0.30	3.22 ± 0.91	2.70 ± 0.49
5 %w/w polymer (D)	4.20 ± 0.73	2.68 ± 0.62	4.27 ± 0.83	3.78 ± 0.70
ANOVA	P < 0.05	P > 0.05 (0.18)	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & D		A & D B & D	A & D

Appendix 30 : The peak force of detachment (F_{max}) of SS suppositories measured using tensile setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	1.82 ± 0.13	1.82 ± 0.13	1.82 ± 0.13	1.82 ± 0.13
1 %w/w polymer (B)	2.68 ± 0.51	1.90 ± 0.49	2.91 ± 0.64	2.24 ± 0.25
2 %w/w polymer (C)	3.80 ± 0.17	2.27 ± 0.50	3.53 ± 0.45	2.79 ± 0.33
5 %w/w polymer (D)	4.57 ± 0.29	2.86 ± 0.50	4.94 ± 0.65	3.56 ± 0.22
ANOVA	P < 0.05	P > 0.05 (0.07)	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & C A & D B & C B & D		A & C A & D B & D C & D	A & D

Appendix 31 : The peak force of detachment (F_{max}) of CB suppositories measured using shear setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04
1 %w/w polymer (B)	0.37 ± 0.02	0.43 ± 0.07	0.51 ± 0.03	0.57 ± 0.02
2 %w/w polymer (C)	0.41 ± 0.07	0.55 ± 0.03	0.55 ± 0.06	0.58 ± 0.03
5 %w/w polymer (D)	0.57 ± 0.05	0.63 ± 0.05	0.85 ± 0.01	0.71 ± 0.02
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & D B & D C & D	A & B A & C A & D B & C B & D	A & B A & C A & D B & D C & D	A & B A & C A & D B & D C & D

Appendix 32 : The peak force of detachment (F_{max}) of CE suppositories measured using shear setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	0.46 ± 0.02	0.46 ± 0.02	0.46 ± 0.02	0.46 ± 0.02
1 %w/w polymer (B)	0.49 ± 0.04	0.56 ± 0.05	0.52 ± 0.05	0.56 ± 0.04
2 %w/w polymer (C)	0.49 ± 0.01	0.56 ± 0.01	0.69 ± 0.06	0.63 ± 0.02
5 %w/w polymer (D)	0.53 ± 0.04	0.56 ± 0.03	0.81 ± 0.07	0.65 ± 0.02
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & D	A & B A & C A & D	A & C A & D B & C B & D C & D	A & B A & C A & D B & D

Appendix 33 : The peak force of detachment (F_{max}) of SS suppositories measured using shear setup against colon mucosa. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION			
	CBP	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.03
1 %w/w polymer (B)	0.40 ± 0.05	0.46 ± 0.04	0.45 ± 0.07	0.48 ± 0.05
2 %w/w polymer (C)	0.49 ± 0.05	0.49 ± 0.04	0.65 ± 0.04	0.53 ± 0.04
5 %w/w polymer (D)	0.59 ± 0.03	0.55 ± 0.04	0.78 ± 0.05	0.61 ± 0.04
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & D B & D C & D	A & D B & D C & D	A & C A & D B & C B & D C & D	A & C A & D B & D C & D

Appendix 34 : The peak force of detachment (F_{max}) of SS suppositories measured using tensile setup against colon mucosa and synthetic regenerated cellulose. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION							
	Colon mucosa				**Synthetic membrane**			
	CBP	**HPMC**	**PVP**	**CMCTS**	**CBP**	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	1.82 ± 0.13	1.82 ± 0.13	1.82 ± 0.13	1.82 ± 0.13	9.89 ± 0.86	9.89 ± 0.86	9.89 ± 0.86	9.89 ± 0.86
2 %w/w polymer (B)	3.80 ± 0.17	2.27 ± 0.50	3.53 ± 0.45	2.79 ± 0.33	12.00 ± 1.19	11.92 ± 0.85	13.13 ± 1.41	12.98 ± 0.73
5 %w/w polymer (C)	4.57 ± 0.29	2.86 ± 0.50	4.94 ± 0.65	3.56 ± 0.22	13.71 ± 0.48	12.60 ± 1.27	14.60 ± 0.48	13.69 ± 0.42
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD	A & C	A & C	A & B	A & C	A & C	A & C	A & B	A & B
SIGNIFICANT	A & B		A & C	A & B			A & C	A & C
DIFFERENCE	B & C		B & C	B & C				

Appendix 35 : The peak force of detachment (F_{max}) of SS suppositories measured using shear setup against colon mucosa and synthetic regenerated cellulose. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=5-6.

	FORMULATION							
	Colon mucosa				**Synthetic membrane**			
	CBP	**HPMC**	**PVP**	**CMCTS**	**CBP**	**HPMC**	**PVP**	**CMCTS**
DcNa only (A)	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	0.40 ± 0.07	0.40 ± 0.07	0.40 ± 0.07	0.40 ± 0.07
2 %w/w polymer (B)	0.49 ± 0.05	0.49 ± 0.04	0.65 ± 0.04	0.53 ± 0.04	0.47 ± 0.06	0.47 ± 0.04	0.54 ± 0.05	0.53 ± 0.02
5 %w/w polymer (C)	0.59 ± 0.03	0.55 ± 0.04	0.78 ± 0.05	0.61 ± 0.04	0.53 ± 0.04	0.53 ± 0.05	0.70 ± 0.07	0.62 ± 0.07
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD	A & C	A & C	A & B	A & B	A & C	A & C	A & B	A & B
SIGNIFICANT	B & C	B & C	A & C	A & C			A & C	A & C
DIFFERENCE			B & C				B & C	

Appendix 36 : Hardness values (N) of suppositories subjected to various storage condition and duration. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=6.

	FORMULATION					
	CB + DcNa + 5 % PVP	CB + DcNa + 5 % CMCTS	CE + DcNa + 5 % PVP	CE + DcNa + 5 % CMCTS	SS + DcNa + 5 % PVP	SS + DcNa + 5 % CMCTS
Fresh samples (A)	99.83 ± 2.32	131.17 ± 2.32	91.33 ± 3.94	106.67 ± 8.09	91.33 ± 3.93	106.67 ± 8.09
Refrigerated for 100 days (B)	133.83 ± 5.64	114.50 ± 2.80	118.67 ± 5.05	106.50 ± 4.09	108.67 ± 5.05	106.50 ± 4.09
Refrigerated for 200 days (C)	128.33 ± 3.51	138.33 ± 5.32	127.67 ± 4.27	116.67 ± 8.71	100.83 ± 8.35	114.17 ± 2.93
Room temperature for 100 days (D)	116.17 ± 1.60	127.67 ± 4.27	98.17 ± 6.55	111.17 ± 7.83	82.83 ± 6.05	111.17 ± 7.83
Room temperature for 200 days (E)	123.50 ± 5.05	131.17 ± 3.87	112.67 ± 6.44	102.83 ± 7.78	86.83 ± 5.23	106.17 ± 2.79
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C	B & D B & E C & E D & E	A & C B & C C & D C & E	A & B A & C A & E B & D	C & D C & E D & E	C & E	A & B A & C B & C B & D B & E	C & E	A & C B & C C & D

Appendix 37 : Softening time (min) of suppositories subjected to various storage condition and duration. Outcomes were a result of ANOVA and post hoc Tukey's HSD analysis. Mean ± SD, n=3.

	FORMULATION					
	CB + DcNa + 5 % PVP	CB + DcNa + 5 % CMCTS	CE + DcNa + 5 % PVP	CE + DcNa + 5 % CMCTS	SS + DcNa + 5 % PVP	SS + DcNa + 5 % CMCTS
Fresh samples (A)	4.03 ± 0.02	4.20 ± 0.03	5.58 ± 0.08	4.92 ± 0.09	5.13 ± 0.05	5.29 ± 0.04
Refrigerated for 100 days (B)	4.63 ± 0.08	3.99 ± 0.14	4.54 ± 0.04	4.45 ± 0.55	4.77 ± 0.06	4.20 ± 0.64
Refrigerated for 200 days (C)	4.27 ± 0.09	4.03 ± 0.06	5.44 ± 0.08	5.04 ± 0.04	5.14 ± 0.60	4.99 ± 0.06
Room temperature for 100 days (D)	6.38 ± 0.04	6.18 ± 0.13	6.06 ± 0.09	6.24 ± 0.26	5.57 ± 0.05	6.06 ± 0.23
Room temperature for 200 days (E)	6.87 ± 0.09	5.99 ± 0.07	7.08 ± 0.41	7.54 ± 0.06	6.5 ± 0.08	6.33 ± 0.08
ANOVA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
TUKEY'S HSD SIGNIFICANT DIFFERENCE	A & B A & C A & D A & E B & C	B & D B & E C & D C & E D & E	A & D A & E B & D B & E C & D	C & E	A & B A & E A & D B & C B & D	B & E C & D C & E D & E	A & D A & E B & D B & E C & D	C & E	A & E B & D B & E C & E D & E	A & B A & C A & E B & D B & E	C & D C & E